Differential Roles of Neuronal Activity in the Supplementary and Presupplementary Motor Areas: From Information Retrieval to Motor Planning and Execution

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Hoshi, Eiji and Jun Tanji. Differential roles of neuronal activity in the supplementary and presupplementary motor areas: from information retrieval to motor planning and execution. J Neurophysiol 92: 3482–3499, 2004. First published July 21, 2004; doi:10.1152/jn.00547.2004. We explored functional differences between the supplementary and presupplementary motor areas (SMA and pre-SMA, respectively) systematically with respect to multiple behavioral factors, ranging from the retrieval and processing of associative visual signals to the planning and execution of target-reaching movement. We analyzed neuronal activity while monkeys performed a behavioral task in which two visual instruction cues were given successively with a delay: one cue instructed the location of the reach target, and the other instructed arm use (right or left). After a second delay, the monkey received a motor-set cue to be prepared to make the reaching movement as instructed. Finally, after a GO signal, it reached for the instructed target with the instructed arm. We found the following apparent differences in activity: 1) neuronal activity preceding the appearance of visual cues was more frequent in the pre-SMA; 2) a majority of pre-SMA neurons, but many fewer SMA neurons, responded to the first or second cue, reflecting what was shown or instructed; 3) in addition, pre-SMA neurons often reflected information combining the instructions in the first and second cues; 4) during the motor-set period, pre-SMA neurons preferentially reflected the location of the target, while SMA neurons mainly reflected which arm to use; and 5) when executing the movement, a majority of SMA neurons increased their activity and were largely selective for the use of either the ipsilateral or contralateral arm. In contrast, the activity of pre-SMA neurons tended to be suppressed. These findings point to the functional specialization of the two areas, with respect to receiving associative cues, information processing, motor behavior, planning, and movement execution.

INTRODUCTION

The supplementary (SMA) and presupplementary (pre-SMA) motor areas, which are located in area 6 on the medial wall of the frontal cortex of primates, are separate motor areas with distinct structural and functional properties (Picard and Strick 1996; Tanji 1996). Anatomical studies revealed differences in the connectivity of the two areas. Only the SMA has direct connections with the primary motor cortex and descending output to the spinal cord (Dum and Strick 1991; Maaheron et al. 1982; Maier et al. 2002), whereas the pre-SMA receives afferents from the dorsolateral prefrontal cortex (Lu et al. 1994; Luppino et al. 1993). Thalamic projections to the two areas come from largely separate areas (Matelli and Luppino 1996). The SMA is organized somatotopically in the caudal to rostral direction (Matsuzaka and Tanji 1996; Tanji 1994). Intracortical microstimulation evokes body movements, allowing construction of a somatotopic motor map (Luppino et al. 1991; Mitzi and Wise 1987). In contrast, for the pre-SMA, microstimulation of this area had complex effects involving the forelimb (Luppino et al. 1991). Ablation of the SMA produces specific deficits in bimanual coordination (Brinkman 1984) or internally guided or instructed movements (Chen et al. 1995; Kazennikov et al. 1998; Kermadi et al. 1997; Thaler et al. 1995), whereas chemical inactivation causes the failure of the sequential execution of multiple movements (Shima and Tanji 1998). Clinical studies reported comparable impairments with SMA lesions: the failure of sequential motor performance (Laplane et al. 1977) or a reduction in spontaneous movements (Krainik et al. 2001). On the other hand, lesions of the pre-SMA caused deficits in updating sequential movements (Shima and Tanji 1998) and the acquisition of sequential procedures (Nakamura et al. 1999). Brain imaging studies in humans have implicated the pre-SMA in motor-task learning (Friston et al. 1992; Hikosaka et al. 1996) and in performing motor tasks with higher cognitive demands (Deiber et al. 1991; Sergent et al. 1992; Zatorre et al. 1994).

Studies examining single-cell activity have pointed to the different roles these two areas play in controlling motor behavior. First, it was found that SMA neurons exhibited marked activity during an arm movement, whereas pre-SMA neurons were more active in response to visual signals or during a preparatory period (Matsuzaka et al. 1992). Second, with a motor task requiring subjects to switch the direction of forthcoming reaching movement, neurons that were selectively active when shifting the direction of action occurred more often in the pre-SMA than in the SMA (Matsuzaka and Tanji 1996). Third, neuron activity selectively associated with capturing a spatial target with either a saccade or an arm reach, i.e., neurons involved in effector-independent reaching, was more frequent in the pre-SMA (Fujii et al. 2002). Finally, with sequential movement tasks, it was found that J) SMA neurons were more active when memory guided a sequence of movements (Mushiake et al. 1991; Tanji and Shima 1994), whereas pre-SMA neurons were more active when visual signals guided the sequence (Halsband et al. 1994); 2) neuron activity during...
the linking of two different movements was found more often in the SMA (Shima and Tanji 2000; Tanji 2001), whereas neurons selectively active when updating sequential order were more frequent in the pre-SMA (Shima et al. 1996); 3) neurons selective for the numerical order were more frequent in the pre-SMA (Clower and Alexander 1998; Shima and Tanji 2000); and 4) neuronal activity during the acquisition of sequential movements were more abundant in the pre-SMA (Nakamura et al. 1998; however, see Lee and Quesy 2003).

Despite these reports, more studies of neuronal activity relevant to other aspects of behavioral processes are still necessary. Recent brain imaging studies in humans suggest that the pre-SMA participates in another aspect of behavioral control. Two studies reported that the pre-SMA was active in conditional motor behavior in which auditory or visual signals were associated with the selection of finger movements (Kurata et al. 2000; Sakai et al. 2000). These reports suggest that the pre-SMA, but not the SMA, is involved in mapping sensory signals to movements (Picard and Strick 2001), inviting studies to analyze how sensory signals are received, processed, and transformed into information useful for motor selection at the single-cell level. On the other hand, the nature of preparatory activity preponderant in both the SMA and pre-SMA remains to be clarified (Matsuzaka et al. 1992; Tanji 1996).

To address these issues, we devised a behavioral task that separated each step in the information processing of the visuo-motor transformation required to associate visual signals with movements. In the initial part of the task, two instructions indicating arm use and target location were given successively with a delay between them, requiring the subjects to detect sensory signals and to extract necessary information. Subsequently, information had to be integrated to generate information to plan forthcoming actions. Then, a motor-set period was introduced to sort the preparatory processes before initiating a reaching movement in response to a trigger signal. We will show that neurons in the SMA and pre-SMA exhibit differential properties with respect to receiving associative cues, retrieving and integrating information, planning motor behavior, and executing movements.

METHODS

Animals and apparatus

We studied two male monkeys (Macaca fuscata, 8 kg) that were cared for in accordance with the National Institutes of Health guidelines and the Guidelines for Animal Care and Use published by our institute. The two monkeys were also used in previous studies (Hoshi and Tanji 2000, 2002, 2004). During the experimental sessions, each monkey sat in a chair with its head restrained. We installed two touch-pads (17 cm apart) in front of the chair, and a color monitor was placed in front of the monkey (30 cm from its eyes). Eye positions were monitored with an eye-camera system (R-21C-AS, RMS, Hirosaki, Japan). Neuronal activity was recorded with glass-insulated Elgiloy-alloy micro-electrodes (1 ~ 2 MΩ at 333 Hz), which were inserted through the dura mater using a hydraulic microdrive (MO-81, Narishige, Tokyo, Japan). Single-unit potentials were amplified with a multichannel processor and sorted using a multispikes detector (MCP plus 8, MSD, Alpha Omega Engineering, Nazareth, Israel). Neuronal activity was recorded with glass-insulated Elgiloy-alloy micro-electrodes (1 ~ 2 MΩ at 333 Hz), which were inserted through the dura mater using a hydraulic microdrive (MO-81, Narishige, Tokyo, Japan). Single-unit potentials were amplified with a multichannel processor and sorted using a multispikes detector (MCP plus 8, MSD, Alpha Omega Engineering, Nazareth, Israel). EMG activity was recorded with silver wire electrodes. The EMG activity was amplified and digitized with an A/D converter, and the digital values were stored in a laboratory computer. The TEMPO/Win system (Reflective Computing, St. Louis, MO) controlled the behavioral task and saved data for off-line analysis.

Behavioral task

The monkeys were trained to perform a target-reach task by following two sets of instructions, one indicating the target location and the other indicating which arm to use to reach for the target (Fig. 1A). The task commenced when the monkey placed a hand on each touch-pad, after an inter-trial interval of ≥3 s, and gazed at a fixation point (FP) 1.2° in diameter that appeared at the center of the touch-sensitive screen. If fixation was maintained for 1,200 ms, the monkey was given the first instruction (1st cue, 400-ms duration), which contained information about either the target location or which arm to use. A small, colored cue that was superimposed on the central FP indicated the type of instruction, i.e., whether the instruction was related to the target location or the arm to use. For monkey 1, a green
circle or red square indicated the instruction for arm use, whereas a blue circle or red cross indicated the instruction for target location. For monkey 2, a green square and blue cross indicated the instruction for arm use and target location, respectively. A white square (8 × 8") that appeared to the left or right of the FP, appearing at the same time as the colored cue, indicated the laterality of arm use (for the arm instruction) or target location (for the target instruction). If fixation was maintained for 1,200 ms during the subsequent delay period (1st delay), the second instruction (2nd cue, 400 ms) was given to complete the information for the subsequent action. Thereafter, if fixation was maintained for 1,200 ms during the second delay, squares appeared on each side of the FP (set cue, ≥1,000 ms), telling the monkey to get ready 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 was rewarded with fruit juice. Before the GO signal appeared, monkey 1 was required to fixate on the FP for 800 – 1,200 ms. 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 the order of instructions.

Data analysis

DEFINITION OF TASK-RELATED NEURONS AND 10 TASK PERIODS. We sampled all neurons that were monitored during at least four blocks of trials (i.e., 80 trials). For the purpose of defining neuronal activity as task-related, we initially divided the behavioral task into the following six periods: 1) control, 200 – 700 ms after attaining fixation; 2) prefirst cue, the 500-ms period before the first cue appeared; 3) first cue and delay, from 100 ms after the first cue onset until onset of the second cue; 4) second cue and delay, from 100 ms after onset of the second cue until onset of the set cue; 5) set cue, from onset of the set cue until the GO signal appeared; and 6) movement, the 500-ms period around the time when movement started. We classified a neuron as "task-related" if the distribution of the discharge rate (spikes/s) was significantly different in at least one of eight trial types (ANOVA, P < 0.05, repeated over 8 trial types with 8 sequences of the 1st and 2nd cues). For the purpose of statistical analysis and display, data were aligned separately for the five task events (onsets of the 1st and 2nd cues, the set cue, GO, and the time of screen touch). These data were analyzed separately before being merged at the midpoint of the first and second delays and of the set-cue period (i.e., 600 ms after cue onset and 600 ms before the onset of the 2nd cue or the set cue, and 600 ms after the set-cue onset and 600 ms before the GO signal).

Subsequently, for the purpose of examining properties of neuronal activity with statistical analyses, we divided the total task phases into one “control period” (200–700 ms after attaining fixation) and 10 “task periods,” as follows: 1) prefirst cue, 500-ms period before the onset of the first cue; 2) first cue, 100 – 500 ms after the onset of the first cue; 3) early first delay, 500 – 1,000 ms after the onset of the first cue; 4) late first delay, last 500 ms before the onset of the second cue; 5) second cue, 100 – 500 ms after the onset of the second cue; 6) early second delay, 500 – 1,000 ms after the onset of the second cue; 7) late second delay, last 500 ms before the onset of the set cue; 8) early set-cue, 500-ms period after the onset of the set cue; 9) late set-cue, 500 ms before GO appeared; and 10) movement, 500-ms period before the screen was touched.

STATISTICAL ANALYSIS USING INTERSPIKE INTERVALS. To analyze neuronal activity with high temporal resolution, we first calculated the instantaneous firing rate as the inverse of the interspike interval (inverse-ISI, 1-ms resolution). Since 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 how neuronal activity reflected information contained in the first, second, or both cues, we used a one-way ANOVA. We examined how well each of the following formulas expressed neuronal activity

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

(1)

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

(2)

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

(3)

In these formulas, the firing rate index is for the transformed inverse-ISI data that were sampled every 10 ms, \(\beta_0\) is the intercept, and \(\beta_a\), \(\beta_b\), and \(\beta_c\) are coefficients. The categorical factors for the first and second CUE are the four instructions provided in the cues (right arm, right target, left arm, and left target). The categorical factors for COMBINATION are the four possible combinations of arm use and target location given by the first and second cues. First, we calculated the probability (P value) that the coefficient of each formula equaled zero. We calculated P values for each 10-ms time-point (i.e., bin) using a custom-made algorithm that was executed with commercially available software (MATLAB 6.5, MathWorks, Natick, MA). We took P < 0.01 to be statistically significant. Then, we calculated the sum of squares (SS) between groups and divided this value by the total SS to obtain the SS ratio. These SS values were obtained from ANOVA tables using a custom-made algorithm that was executed with commercial software (MATLAB 6.5, MathWorks). The SS ratio was analyzed for each 10-ms bin of data. The larger the SS ratio, the better the firing rate index formula represented neuronal activity. Based on the analysis of probability and the SS ratio, we classified neurons into four categories, according to whether the instantaneous activity was best and significantly represented by 1) the first cue, 2) the second cue, 3) the combination of arm target information, or 4) none of the regression coefficients were significantly different from zero. This classification was carried out for every 10-ms bin.

LINEAR MODEL ANALYSIS AFTER THE APPEARANCE OF THE SECOND CUE. For activity after the second cue appeared, we quantified the extent to which neuronal activity represented selectivity for the second cue or the combination of the first and second cues. We used the following linear model to execute an ANOVA analysis

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

(4)

In this formula, the firing rate index is the square-root transformed firing rate during the second delay period, \(\beta_0\) is the intercept, and \(\beta_a\) and \(\beta_c\) are coefficients. The categorical factors for the second cue (CUE2) are the four instructions given by the second cue (right arm, right target, left arm, and left target). The categorical factors for the combination of both the first and second cues (COMBINATION) are the four possible combinations of the two instructions indicating arm use and target location. We classified the neurons into four groups by looking at the probability that coefficients \(\beta_a\) and \(\beta_c\) were zero: 1) combination-only-selective group (\(P_{\beta_1} = 0 \geq 0.01\) and \(P_{\beta_2} = 0 < 0.01\)), 2) second-cue-only-selective group (\(P_{\beta_1} = 0 < 0.01\) and \(P_{\beta_2} = 0 \geq 0.01\)), 3) selective-for-both group (\(P_{\beta_1} = 0 < 0.01\) and \(P_{\beta_2} = 0 < 0.01\)), and 4) nonselective group (\(P_{\beta_1} = 0 \geq 0.01\) and \(P_{\beta_2} = 0 \geq 0.01\)).

QUANTIFICATION OF SELECTIVITY FOR ARM USE AND TARGET LOCATION. To examine the extent to which individual neurons exhibited selectivity for 1) the location of the target or 2) arm use during the set-cue and movement periods, we applied a multiple regression analysis using the following model formula

\[
\text{Firing rate} = \beta_0 + \beta_a \times (\text{left-right target location}) + \beta_b \times (\text{left-right arm use})
\]

(5)

The default values of the variables for right and left were 0 and 1, respectively. We calculated the values of slopes \(\beta_a\) and \(\beta_b\) (by dividing the difference in activity in spikes/s by the dimensionless initial variable values 1 and 0) to assess target location and arm use selectivity, respectively. If \(\beta_i > 0\), it meant greater selectivity for the
left target. If \( \beta_2 > 0 \), the selectivity was greater for the left arm. A merit of this analysis was that we could measure the selectivity with the dimension of firing rate (spikes/s).

**Quantification of Muscle Activity.** To quantify the activity of muscles during movement execution, we calculated two indexes, the arm index and target index, based on the rectified EMG averaged over 20 trials for each movement. The indexes are defined as follows:

\[
\text{Arm Index} = [(V_{RA-RT} + V_{LA-LT}) - (V_{RA-RT} + V_{LA-LT})] / (V_{RA-RT} + V_{LA-LT}) + V_{RA-RT} + V_{LA-LT})
\]

(6)

\[
\text{Target Index} = [(V_{RA-LT} + V_{RA-RT}) - (V_{RA-LT} + V_{RA-RT})] / (V_{RA-RT} + V_{LA-LT}) + V_{RA-RT} + V_{RA-RT})
\]

(7)

In the formulas, \( V \) is the integrated value of the rectified EMG during the movement period, categorized using the appropriate subscript (RA, right arm; LA, left arm; RT, right target; LT, left target). Therefore \( V_{RA-RT} \) means muscle activity while reaching to the right target with the right arm. The indexes, which range from \(-1\) to \(+1\), include information on laterality (positive for “left” preference).

**RESULTS**

**Sampling neuronal activity in the pre-SMA and SMA**

Before we started collecting neuronal activity, we mapped the somatotopic organization of the SMA in the medial wall of the superior frontal gyrus using intracortical microstimulation (ICMS; 11–44 pulses; 200-μs width at 333 Hz; current, 5–50 μA) and by observing neuronal responses to the somatosensory stimuli applied by the experimenters. From caudal to rostral in the SMA, we found somatotopic representation arranged in the order leg, hip, trunk, arm, and face, as reported previously (Luppino et al. 1991; Matsuzaka et al. 1992; Mitz and Wise 1987). In the area rostral to the face area of the SMA, the ICMS effects required longer pulse trains, and neuronal responses to the somatosensory stimuli were weaker and less frequent. Instead, we observed ample visual responses to moving objects. We defined this area as the pre-SMA (Matsuzaka and Tanji 1996; Matsuzaka et al. 1992). The recording sites were reconstructed histologically using iron deposition produced by passing a positive DC current through the tips of the micro-electrodes.

We recorded neuronal activity in the two areas (Fig. 1, B and C) in the right hemisphere. We monitored activity of every neuron we encountered by making peri-event rastergrams aligned at several task events. If the activity was judged to be modified by the appearance of any events, we continued to record the activity for off-line analysis. We analyzed 329 SMA neurons (148 in monkey 1 and 181 in monkey 2) and 349 pre-SMA neurons (107 in monkey 1 and 242 in monkey 2) that were found to be task-related (see Methods). One-third of pre-SMA neurons we encountered and monitored on-line were found task-related with the off-line analysis. During recording, the success rate for the behavioral task was >96% for both monkeys.

**Frequency of a change in activity during each task period**

To study the overall changes in neuronal activity, we analyzed how many neurons in the pre-SMA and SMA showed increased or decreased activity during each of the 10 task periods (see Methods). For each task period, we applied a paired \( t \)-test for each of the eight sequences of the first and second cues (paired \( t \)-test, \( \alpha = 0.05 \), corrected for 8 repeated analyses) compared with the control period. The results are summarized in Fig. 2. The fractions of neurons showing increased or decreased activity are depicted with thin solid and dotted lines, respectively. The bold line indicates the fraction of neurons with either increased or decreased activity (a neuron can show an increase in one trial type and a decrease in another, thus the sum of the fractions can exceed the total number of neurons). In the pre-SMA (Fig. 2A), >30% of the neurons exhibited changes in activity in the 10 task periods, most markedly in the second cue period, when 231 neurons (66%) showed changes (Fig. 2A, ▼). In contrast, in the SMA (Fig. 2B), the fraction of activity-modulated neurons remained low before the onset of the set cue and rose sharply toward the initiation of the reaching movement; 227 neurons (68%) changed their activity (Fig. 2B, ▼). The \( \chi^2 \)-test revealed that a change in activity was more frequent among pre-SMA neurons from the pre-cue to the late set-cue periods (\( P < 0.001 \) for each comparison), whereas activity changes during the movement period were more frequent in the SMA (\( P < 0.0001 \)).

**FIG. 2.** Time courses of the change in neuronal activity in the pre-SMA (A) and SMA (B) over all the task periods. Thick lines indicate the fraction of neurons whose activity increased or decreased significantly compared with activity during the control period. Thin solid and dotted lines represent the fractions of neurons whose activity increased or decreased compared with activity during the control period, respectively. Gray areas indicate task periods during which visual cues were presented (from left to right: 1st cue, 2nd cue, and set cue). *** \( P < 0.0001 \), ** \( P < 0.001 \), * \( P < 0.01 \); task phase during which the fraction of neurons was larger in the pre-SMA or SMA. Numbers at the top of each panel are the actual numbers of neurons that changed activity (i.e., increase or decrease).
Preponderance of precue anticipatory activity in the pre-SMA

Before the first cue appeared (i.e., the precue period), 107 (30%) neurons in the pre-SMA showed significant modification of their activity compared with activity during the control period (paired t-test, \( \alpha = 0.05 \)). This modification resembled that reported in the dorsal premotor cortex as activity anticipating the appearance of the cue (Mauritz and Wise 1986). In contrast, only 20 (6%) neurons in the SMA showed this modification. The frequencies of occurrence of the anticipatory activity differed significantly between the two areas (Fig. 2; Pearson’s \( \chi^2 \) test with Yates’ continuity correction, \( \chi^2 = 65.609, df = 1, P < 0.00001 \)). Precue anticipatory activity was also found in 39 (11%) pre-SMA and 7 (2%) SMA neurons before the second cue. Furthermore, we found that 79 (22%) pre-SMA and 22 (6%) SMA neurons exhibited precue anticipatory activity before the set cue. The anticipatory activity before the second- and set-cue was counted 1) if the activity during the late delay period differed from that in the early delay period (paired t-test, \( \alpha = 0.01 \)) and 2) if the activity did not reflect specific information given by the cues (\( P > 0.01 \) in every factor analysis). An example of pre-SMA neurons showing anticipatory activity is shown in Fig. 3. This neuron showed build-up activity before the appearance of the first, second, and set cues.

The anticipatory activity seen before the first cue, which was commonly found in the pre-SMA, could reflect a behavioral rule or a specific expectation for the appearance of an arm or target instruction because the two sets of instructions (i.e., arm use or target location) were given in a fixed order for a block of 20 trials. To study this possibility, we applied a two-sample \( t \)-test to the activity in the precue period with the order of instructions as the factor. Of the 107 neurons with anticipatory activity in the pre-SMA, only 6 neurons (5%) showed significant differences (2-sample \( t \)-test, \( \alpha = 0.01 \)). Thus this result indicated that only a small part of the anticipatory precue activity in the pre-SMA encodes a behavioral rule or the specific expectation of forthcoming cues.

Neuronal activity in the pre-SMA and SMA after the first cue

After the first cue was presented, >40% of pre-SMA neurons exhibited significant changes in activity compared with activity during the control period \( n = 173 \) (49%) during the first-cue period, \( n = 153 \) (43%) during the early delay, and \( n = 168 \) (48%) for the late delay]. In contrast, fewer SMA neurons responded to the first cue \( n = 50 \) (15%), 46 (13%), and 54 (16%) during the cue, early delay, and late delay periods, respectively. The distributions of neurons with activity changes in the three periods differed significantly (Fig. 2; \( \chi^2 \) test, \( P < 0.0001 \)).

We found that the activity properties of pre-SMA neurons could be grouped into three different types. The first type reflected the position of the white square in the first visual cue. An example of preferential activity for the position of the white square is shown in Fig. 4. The neuron was distinctly more active when the first cue was for either the left target or left arm than when the cue was for the right target or right arm. The common factor in the signals that led to an increase in neuronal activity was the appearance of the white square on the left. The second type of activity reflected the fact that the first cue contained an instruction for the location of the target. An example of a neuron of this type is shown in Fig. 5, where the first delay activity was selective for the right-side target. The third type of activity reflected the fact that the first cue had indicated which arm to use. In the example shown in Fig. 6, the pre-SMA neuron responded phasically to the cue that instructed the use of the right “arm.” However, its activity was suppressed after the right “target” instruction. During the first delay period, for this particular neuron, the activity was more vigorous when the cue instructed the use of the left “arm” than left “target.” Statistical tests supported these points. During the cue period, activity was significantly modified by two factors: type of instruction and position of the white square (2-way ANOVA, \( P < 0.0001 \) for main factors of type of instruction and the position of the white square). Furthermore, activity after the “right arm” instruction was significantly larger than activity after the other three instructions (\( P < 0.0001 \), Bonferroni pairwise comparisons). Similarly, during the early and late delay periods, activity was significantly modified by the two factors, and the activity was more vigorous when the cue instructed the “left arm use” than when instructed other three instructions (\( P < 0.004 \), Bonferroni pairwise comparisons).
To analyze how information given by the first cue was represented by the activity of pre-SMA and SMA neurons systematically, we applied two-way ANOVA with two categorical factors: the type of INSTRUCTION (arm use or target location) and the POSITION of the white square (left or right).

Activity during each of the three task periods, i.e., the cue, early delay, and late delay periods, was analyzed separately. We applied this analysis to the neurons whose activity was modified significantly during each period (Fig. 2). The number and proportion of neurons showing significant selectivity for the POSITION, INSTRUCTION, or both are displayed in Fig. 7. It is apparent that a greater number and proportion of pre-SMA neurons exhibited selectivity to POSITION and INSTRUCTION than of SMA neurons. The differences were significant for the activity throughout the cue, early delay, and late delay periods ($\chi^2$ test, $P < 0.0001$).

To analyze the activity properties of pre-SMA and SMA neurons at a higher temporal resolution, we calculated the fraction of neurons that displayed position selectivity or instruction selectivity for each 10-ms bin during the precue, first cue, and first delay periods using the inverse- ISI data (see METHODS). The results are summarized in Fig. 8. In the pre-SMA (Fig. 8, top left), the fraction of neurons that was selective for the spatial position of the white square (2-way ANOVA, $P < 0.01$ for POSITION or $P < 0.01$ for POSITION × INSTRUCTION) rose to 30% during the 400-ms cue period, and the fraction remained >20% throughout the delay period. In the SMA (Fig. 8, bottom), few neurons were selective for the spatial position. The solid line in the Fig. 8, left, denotes the fraction of cue-position-selective neurons in each 10-ms bin (2-way ANOVA, $P < 0.01$ for POSITION or $P < 0.01$ for POSITION × INSTRUCTION). The fraction of position-selective neurons was greater in the pre-SMA in 151 of 160 10-ms bins during the cue and delay periods ($\chi^2$ goodness-of-fit test with Yates' continuity correction, $\alpha = 0.01$). Of the position-selective neurons in the pre-SMA, 70% were classified as selective for position only and were not
selective for the type of instruction (Fig. 8, top left, dotted line; 2-way ANOVA, \( P < 0.01 \) for POSITION, \( P > 0.01 \) for INSTRUCTION). Subsequently, we analyzed INSTRUCTION selectivity quantitatively (arm use vs. target location). We calculated the fraction of task-related neurons in the pre-SMA and SMA that was selective for the type of instruction. The results are summarized in Fig. 8, right. Neurons that were selective for the type of instruction (solid line, 2-way ANOVA, \( P < 0.01 \) for INSTRUCTION or \( P < 0.01 \) for POSITION \( \times \) INSTRUCTION) were observed mainly in the pre-SMA. We found that 47% of the instruction-selective neurons in the pre-SMA (average during the cue and delay periods) responded preferentially to instructions concerning which arm to use (dotted line); the remaining neurons (53%) were selective for the target location. An additional statistical test revealed that neurons that were selective for either the target location or for which arm to use were found more frequently in the pre-SMA (\( \chi^2 \) test, \( \alpha = 0.01 \)). In 104 of 160 10-ms bins during the cue and delay periods, the fraction of target instruction-selective neurons was greater in the pre-SMA than in the SMA, whereas the fraction of arm instruction-selective neurons was greater in the pre-SMA in 78 bins.

We analyzed the timing of the onset of changes in neuronal activity in response to the first cue. We defined the onset of cue-selective activity as the time at which the fraction of cue-selective neurons first exceeded 10% of the total population of neurons. In the pre-SMA, the onset of position selectivity was 100 ms, and the onset of instruction selectivity was 230 ms. In the SMA, both position selectivity and instruction selectivity failed to reach the 10% threshold.

![Time course of the selectivity for neuronal activity during the 1st cue and delay periods: bin-by-bin plots of the fraction of neurons exhibiting selectivity for each category. Top: data for the pre-SMA. Bottom: data for the SMA. Left column: position selectivity for the white square. Gray areas in each panel indicate when the cues appeared. Solid lines represent the fraction of neurons that was position-selective, calculated successively for each 10-ms bin. Dotted lines represent the fraction of neurons that exhibited arm or target instruction selectivity. Tic marks on the horizontal axis are placed at 400-ms intervals. Right column: time course of instruction selectivity. Solid lines represent the fraction of neurons that exhibited arm or target instruction selectivity. Dotted lines represent the fraction of neurons that exhibited selectivity for the arm instruction.](http://jn.physiology.org/)}
In summary, for activity during the first cue and delay periods, neurons that were selective for either the position of the cue or the type of instruction were much more numerous in the pre-SMA than in the SMA. Further, the position selectivity in the pre-SMA appeared promptly after the first cue appeared, which was followed by the instruction selectivity 130 ms later.

**Neuronal activity in the pre-SMA and SMA after the second cue**

After the second cue appeared, the number of pre-SMA neurons showing changes in activity reached a peak (as many as 66% of all task-related neurons, see Fig. 2A). In contrast, only 23% of SMA neurons changed their activity in response to the second cue (Fig. 2B). The distributions of neurons with activity changes during the cue, early delay, and late delay periods differed significantly between the two areas ($\chi^2$ test, $\alpha = 0.01$).

We found that the properties of responses to the second cue differed from those to the first cue in both areas. Although some neurons exhibited responses reflecting what the second cue indicated or instructed, these neurons representing the second cue were in the minority. In the majority of cases, we found that the selectivity was not merely for the reflection of the second cue itself. Rather, activity reflected information provided by the combination of two instructions (arm use and target location) given by the first and second cues. In the example shown in Fig. 9, the pre-SMA neuron was active most intensely when the combination of two cues was for the left arm and left target, irrespective of the order of presentation. Statistical tests revealed that activity of the neuron was significantly modified by the two factors of CUE2 and COMBINATION during the early delay period ($P < 0.0001$ for the 2 factors, see Eq. 4 in METHODS) and only by COMBINATION during the late delay period ($P < 0.0001$ for COMBINATION and $P > 0.75$ for CUE2).

We quantified the extent to which neuronal activity in the pre-SMA and SMA was selective for the second cue or the combination of two cues by applying the linear model (Eq. 4) to the activity during the early- and late-delay periods after the second cue. We applied this analysis to all neurons that exhibited significantly modified activity during the early or late second delay periods (see Fig. 2). The distribution of selectivity for the second cue or the combination is summarized in Fig. 10. Neurons with significant combination selectivity ($P_{\beta2} = 0 < 0.01$) or second-cue selectivity ($P_{\beta1} = 0 < 0.01$) were more frequent in the pre-SMA than in the SMA ($\chi^2$ test, $P < 0.005$).

Subsequently, we attempted to visualize the time course of the development of the selectivity of neuronal activity for the first, second, and combination of both cues, together, with high temporal resolution. To do so, we carried out a regression analysis using model Eqs. 1–3. We assigned the activity of each neuron to one of four categories (i.e., significant and most selective for the 1st cue, 2nd cue, the combination of both cues, or nonselective—see METHODS), based on the activity of each neuron in each 10-ms bin. We calculated the fraction of neurons for which activity could be assigned to each of the four categories repeatedly for successive 10-ms bins. In Fig. 11, we plotted, bin-by-bin, the fraction of neurons out of the total number of neurons in the pre-SMA (Fig. 11A) and SMA (Fig. 11B) that were best and significantly selective for the first cue (black traces), second cue (blue traces), and the combination of both cues (red traces). After the first cue appeared, the fraction of neurons selective for the first cue increased in the pre-SMA, but the fraction of neurons in the SMA remained small. After the second cue appeared, the fraction of first-cue-selective pre-SMA neurons decreased promptly, while neurons that were selective for the second cue (blue) or the combination of cues (red) increased (Fig. 11A). Subsequently, the combination-selective neurons soon became dominant. In the SMA (Fig. 11B), there were few second-cue- and combination-selective neurons. We compared the fractions of pre-SMA and SMA neurons that were selective for the first, second, and combination of cues. First-cue-selective neurons were observed more frequently in the pre-SMA during the first cue and delay periods (151 of 160 10-ms bins; $\chi^2$ test, $\alpha = 0.01$). Similarly, second-cue-selective neurons were observed more frequently in the pre-SMA during the second cue and delay periods (92 of 160 bins). Combination-selective neurons were also observed more frequently in the pre-SMA during the second cue and delay periods (151 of 160 10-ms bins).

Finally, we analyzed the timing of the onset of changes in the activity of neurons in response to the second cue. We defined the onset of the selective activity as the time at which the fraction of selective neurons first exceeded 10% of the total population of neurons. In the pre-SMA, the onset of combination selectivity was 120 ms, and the onset of second-cue selectivity was 150 ms. In the SMA, the onset of combination selectivity was 1,530 ms, whereas second-cue selectivity failed.

**Fig. 9.** Activity of a pre-SMA neuron reflecting the combination of 2 instructions, as well as the 2nd cue. This neuron was active most intensely if the combination of the 2 instructions was LA and LT, regardless of the order of the 2 instructions (bottom).
to reach the 10% threshold. Therefore after the second cue appeared, activity reflecting specific combinations of the two instructions developed promptly in the pre-SMA, but not in the SMA.

Neuronal activity in the pre-SMA and SMA during the set-cue period

The appearance of the set cue told the subjects to get ready to reach for the target in response to the GO signal. Throughout the delay period, >60% of the pre-SMA neurons exhibited changes in activity (Fig. 2A). In the SMA, there was a smaller proportion of set-cue-responsive neurons during the early part of the set-cue period (Fig. 2B; $\chi^2$ test, $P < 0.003$). Subsequently, the fraction of set-cue-modulated neurons increased sharply. The results of the quantitative analysis are shown in Fig. 11, based on calculations using Eqs. 1–3. The results revealed that during the set-cue period, most of the activity changes reflected the combination of the two cues (red traces in Fig. 11, A and B). In the pre-SMA, combination selectivity was reflected in 15–20% of the neurons, whereas in the SMA, the combination-selective neurons increased sharply toward the end of the delay, surpassing neurons in the pre-SMA.

A remarkable finding concerning the delay-period activity was that pre-SMA neurons preferentially represented the location of the reach target rather than arm use. A typical example of such target representation is shown in Fig. 12. This shows a pre-SMA neuron in which set-cue period activity was apparent when the target was on the left, regardless of the arm used. In contrast, SMA neurons were more selective for arm use, particularly during the late set-cue period preceding the GO signal. Such activity is exemplified in Fig. 13, where activity
was observed selectively when the subject was prepared to use its left arm.

To systematically analyze the proportion of SMA and pre-SMA neurons reflecting target location or arm use, we applied three-way ANOVA to all neurons whose activities were modified significantly during the set-cue period. The results of this analysis, using the TARGET location, ARM use, and ORDER of instructions, are summarized in Fig. 14. In the pre-SMA, 70 neurons (22%, $P < 0.01$) showed exclusive selectivity for the target during the late set-cue period, whereas only 18 neurons (5%) showed selectivity for arm use. In contrast, in the SMA, 46 neurons (13%) showed selectivity for arm use, and 18 neurons (5%) exhibited selectivity for the target during the same period. Fifteen pre-SMA and 28 SMA (8%) neurons showed selectivity for both arm use and target.

Next, we examined the extent to which individual neurons exhibited selectivity for 1) the location of the target or 2) arm use, by applying a multiple regression analysis (Eq. 5 in METHODS). We applied the analysis to the activity during the early and late set-cue periods if a neuron exhibited significant changes in activity relative to the control period (paired t-test, $\alpha = 0.05$, corrected for 8 trial types). In the pre-SMA, the activity changed significantly in 215 (61%, early set-cue period) and 222 (63%, late set-cue period) neurons. In the SMA, activity changed significantly in 103 (31%, early set-cue period) and 170 (51%, late set-cue period) neurons. For pre-SMA neurons, in both the early and late set-cue periods, target location selectivity (slope $\beta_1$) was greater than arm use selectivity (slope $\beta_2$; Kolmogorov-Smirnov test; KS = 0.2372, $P < 0.001$ for the early set-cue period and KS = 0.2838, $P < 0.001$ for the late set-cue period). Data for the late set-cue period are shown in Fig. 15, A and B. For SMA neurons, the target location (slope $\beta_1$) and arm use (slope $\beta_2$) selectivity did not differ during the early set-cue period (KS = 0.1456, $P = 0.1822$). In contrast, during the late set-cue period, arm use selectivity exceeded target location selectivity (KS = 0.1706, $P = 0.0123$), as shown in Fig. 16, A and B. Note that arm use selectivity exceeded target location selectivity (KS = 0.1706, $P = 0.0123$), as shown in Fig. 16, A and B. Note that arm use

FIG. 13. Set-related activity of a SMA neuron. This neuron was more active if the subject was prepared to reach with the left arm.

FIG. 14. Arm use and target location selectivity of set- and movement-related activity in the pre-SMA and SMA. In each panel, pie charts summarize the proportion of neurons classified into 4 categories by the 3-way ANOVA analysis (3 factors: arm use, target location, and order of the 2 instructions). Top: data for the pre-SMA. Bottom: data for the SMA. Actual number of neurons in each category is shown next to each segment, and these are identified below the segments. The arm use–only neurons were significant ($P < 0.01$) only for the main factor ARM. The target location–only neurons were significant ($P < 0.01$) only for the main factor TARGET. Both arm use and target location neurons were significant ($P < 0.01$) for both of the main factors or for the interaction between ARM×TARGET.
selectivity distributed in the positive and negative ranges non-differentially, indicating that individual neurons in the right SMA showed selectivity for either left or right arm use. The distribution of data points to the left or right of the scatterplot (Fig. 16A) did not differ (Kolmogorov-Smirnov test, KS = 0.1542, P = 0.2447).

To compare arm use and target location representation between pre-SMA and SMA neurons, we applied the Kolmogorov-Smirnov test to the absolute values of slope $\beta_1$ (target location selectivity) and slope $\beta_2$ (arm use selectivity). During the early set-cue period, arm use selectivity did not differ, whereas target location selectivity was already greater for pre-SMA neurons (KS = 0.2572, P < 0.0001). During the late set-cue period, target location selectivity was greater for pre-SMA neurons (KS = 0.1457, P = 0.03), whereas arm use selectivity was greater for SMA neurons (KS = 0.3082, P < 0.0001).

Neuronal activity during movement execution

In the SMA, 68% of task-related neurons changed their activity during the movement-execution period (Fig. 2B). Of these 227 neurons, 181 (79%) exhibited increased activity. In the pre-SMA, 56% of the task-related neurons showed changes in activity during the movement period (Fig. 2A). Of note, a majority ($n = 132$, 67%) of the 196 movement-related neurons showed decreased activity. In both areas, neuronal activity was selective for information reflecting the combination of Cue 1 or Cue 2, rather than reflecting what Cue 1 or Cue 2 showed or instructed individually (Fig. 11, A and B). Greater selectivity was observed for SMA neurons than for pre-SMA neurons.

We found that a majority of the selectivity exhibited by movement-related SMA neurons reflected arm use, as apparent in the SMA activity shown in Fig. 17. In that example, the activity was intense preceding the reach movement using the left arm. To systematically analyze how selectivity for location and arm use were represented in SMA and pre-SMA neurons, we applied three-way ANOVA to movement-related neurons for the factors TARGET location, ARM use, and ORDER of instructions. In the SMA (Fig. 14, bottom), selectivity for arm use was observed in 126 (38%) neurons ($P < 0.01$ for ARM or $P < 0.01$ for ARM \times TARGET), and selectivity for target location was observed in 65 (19%) neurons ($P < 0.01$ for TARGET or $P < 0.01$ for ARM \times TARGET). In the pre-SMA (Fig. 14, top), 58 neurons (16%) showed selectivity for arm use ($P < 0.01$ for ARM or $P < 0.01$ for ARM \times TARGET), and 56 neurons (16%) showed selectivity for target location ($P < 0.01$ for TARGET or $P < 0.01$ for ARM \times TARGET).

Next, we examined the extent to which individual neurons exhibited selectivity for 1) the location of the target or 2) arm use by applying a multiple regression analysis using Eq. 5. We applied this analysis to all the neurons (227 SMA and 196 pre-SMA) exhibiting a significant change in activity during the 500-ms movement period. For SMA neurons, arm use selectivity (slope $\beta_2$) exceeded target location selectivity (slope $\beta_1$; Fig. 16, C and D; KS = 0.4009, $P < 0.0001$ Kolmogorov-Smirnov test), whereas for pre-SMA neurons, target location selectivity and arm use selectivity did not differ (Fig. 15, C and D; KS = 0.0714, $P = 0.6393$). Furthermore, to compare arm use and target location selectivity between pre-SMA and SMA neurons directly, we
applied the Kolmogorov-Smirnov test to the absolute values of slopes $H_1$ (target location selectivity) and $H_2$ (arm use selectivity). Arm use selectivity was greater for SMA neurons ($KS = 0.3739, P = 0.0001$), while target location selectivity did not differ ($KS = 0.1214, P = 0.0831$).

During the motor execution period, although preference for the contralateral arm was dominant in the SMA (Fig. 16C, Kolmogorov-Smirnov test for the absolute values for positive and negative values of arm preference, $KS = 0.2139, P = 0.0123$), 87 of the 227 neurons (38%) exhibited ipsilateral preference.

Time course of arm use and reach-target representation from cue 2 reception to movement

Figure 11 shows that the representation of information given with either Cue 1 or Cue 2 alone faded after the delay periods. In contrast, the combination of information given by both cues was represented throughout the Cue-2 delay, set-cue, and movement periods. We were interested in the time course of arm use and reach-target representation because both are vital for planning and executing the reach movement. This included the information represented in the period from the reception of Cue 2 until movement execution. Therefore we analyzed the activity of all neurons that were defined as best and significantly selective for the combination of two instructions (Fig. 11, red traces). We applied three-way ANOVA (with the factors target location, arm use, and order of instructions) to a
series of inverse-ISI spike data in every 10-ms bin. For each 10-ms bin, we classified the activity into four categories: 1) selective for arm use (ARM $< 0.01$ or ARM $\times$ TARGET $< 0.01$), 2) selective for target location (TARGET $< 0.01$ or ARM $\times$ TARGET $< 0.01$), 3) selective for both arm use and target location (ARM $< 0.01$ and TARGET $< 0.01$, or ARM $\times$ TARGET $< 0.01$), and 4) nonselective. The results of this analysis are shown in Fig. 18 (Fig. 18A for the pre-SMA and Fig. 18B for the SMA), in which we plot, bin by bin, the fraction of neurons whose activity is assigned to categories 1–3. Blue, green, and black traces represent the fractions of neurons classified as selective for target location, arm use, and both, respectively. Figure 18A shows that the appearance of Cue 2 provides both target and arm information to pre-SMA neurons. Throughout the subsequent preparatory period, target information is maintained, while arm use information decreases. Toward movement initiation, target information decays, rather than increases, in the pre-SMA. In contrast, the level of target representation stays low in the SMA (Fig. 18B). Instead, arm use representation predominates and grows rapidly from the middle of the set-cue period to movement initiation.

**Muscle activity**

In addition to neuronal recordings, we monitored the following muscles bilaterally during task performance: the biceps and triceps brachii, deltoid (anterior, lateral, and posterior heads), trapezius, flexor and extensor carpi radialis, supraspinatus, infraspinatus, pectoralis major, rhomboid, and neck and paravertebral muscles. We found that the 12 forearm muscles, but not the neck or paravertebral muscles, increased their activity in association with movement execution. Despite their movement-associated activity, they did not show consistent changes in activity before actual execution of the movements.

To quantify the activity of the 12 muscles during the movement, we calculated two indexes, the arm index and target index (Eqs. 6 and 7 in METHODS), based on the rectified EMG averaged over 20 trials for each movement.

We examined the distribution of the arm and target indexes. Examples of data recorded from the 12 muscles in the left arm of each monkey (12 $\times$ 2 = 24 muscles) are shown in the scatterplot in Fig. 19A. The target index was distributed around the horizontal line, indicating values close to zero. The arm index was distributed widely in the positive range, indicating left-arm preference. To compare arm and target representations statistically, we applied the Kolmogorov-Smirnov test to the absolute indexes. As shown with black cumulative plots in Fig. 19B, the arm index was much greater than the target index (KS = 0.9583, $P < 0.001$). We performed the same analysis repeatedly on data obtained in 12 sessions, continuing to reach the same conclusion. These results indicate that muscle activity mainly reflected arm use and only represented target location to a small degree. This conclusion was reasonable because the horizontal distance between the two targets was small in our experimental system (55 mm or 10.5°).

As already mentioned, SMA neurons preferentially reflected the arm use rather than the location of the target. Thus it was of interest to directly compare neuronal activity and muscle activity with the same measure. To achieve this, we calculated the arm index and target index for SMA neurons by replacing the magnitude of muscle activity with mean firing rate. The data for SMA neurons are shown with gray cumulative plots in Fig. 19B. It appeared that arm use selectivity of SMA neurons was much smaller than that for muscle activity, while the target location selectivity was as small as for muscles.

**DISCUSSION**

In this study, we observed striking differences in the activity of SMA and pre-SMA neurons with respect to five behavioral aspects. First, neuronal activity preceding the appearance of visual cues was more frequent in the pre-SMA. Second, in response to the appearance of the first instructional cue, a sizeable number of pre-SMA neurons, but few SMA neurons,
were active during the cue and delay periods. Third, neurons responding to the second instructional cue were much more frequent in the pre-SMA. In addition, pre-SMA neurons often reflected information combining the instructions in the first and second cues. Fourth, in response to the set cue’s prompting preparation for a forthcoming movement, pre-SMA neurons preferentially reflected the location of the target. In contrast, SMA neurons mainly reflected which arm to use. Fifth, during the execution of the reaching movement, the majority of SMA neurons increased their activity, which was largely selective for use of either the ipsilateral or contralateral arm. In contrast, the activity of the majority of pre-SMA neurons tended to be suppressed. Based on these findings, we discuss the implications of the differential properties of SMA and pre-SMA neurons, which suggest functional specialization of the two areas, with respect to receiving associative cues, processing information, planning motor behavior, and executing movement.

Pre-cue activity in the pre-SMA

As many as 30% of the task-related neurons in the pre-SMA changed activity before the onset of the instruction cues. This activity is akin to the precise anticipatory activity reported in the dorsal premotor cortex (Mauritz and Wise 1986) and might reflect the anticipation of predictable behavioral events. Is it possible that this activity was selective for the order of appearance of the reach target and arm use instructions? We asked this question because the order of instructions remained unchanged within a task block of 20 trials. We found that 5% of the pre-SMA activity reflected the order of the two instructions, suggesting that a small part of the pre-SMA activity in the pre-SMA reflects the expectation of a specific category of forthcoming cues. Such behavioral context–dependent expectation of cues or the expectation of a specific category of cues has been reported in the dorsal premotor cortex and prefrontal cortex (Sakagami and Niki 1994; Wallis and Miller 2003; White and Wise 1999). One plausible interpretation is that the pre-SMA activity corresponds to the activity previously reported specifically when subjects were in the process of updating the behavioral sequence (Isoda and Tanji 2004; Shima et al. 1996), because our subjects were required to “update” their action at each trial on receiving two visual instructions.

Implication of pre-SMA activity responding to the first visual cue

Pre-SMA neurons responded to the first visual cue much more than SMA neurons did. This observation is in line with differences in visual responsiveness reported in the two areas (Matsuzaka et al. 1992). The visual responses of pre-SMA neurons (Akkal et al. 2002; Rizzolatti et al. 1990) seem to have an anatomical basis. Cortico-cortical connections between the pre-SMA and dorsolateral prefrontal cortex (Bates and Goldman-Rakic 1993; Lu et al. 1994), peri-arcuate premotor areas F5, F7, and rostral F2 (Luppino et al. 1993), or PFG and PG in the inferior parietal lobule (Luppino et al. 1993), as well as thalamocortical connections (Inase et al. 1996; Matelli and Luppino 1996) could provide a route to convey visual information to the pre-SMA. We found two distinct properties of visual responses among pre-SMA neurons. The first reflected the spatial position of the white square in the first cue. This spatial information is likely provided by input from the posterior parietal cortex, the dl-PFC (e.g., Hoshi et al. 2000; Hoshi and Tanji 2004), or the premotor areas (Fogassi et al. 1999; Godschalk et al. 1981; Rizzolatti et al. 1988). The second property of neuronal activity reflected information instructed by the visual cue, rather than visuospatial information, i.e., information about arm use or target location. This property, which already has behavioral significance and constitutes information necessary for planning forthcoming motor behavior, seems to be the outcome of behavior-oriented information processing. This processing might occur in the pre-SMA or elsewhere, e.g., in the dorsal part of the dl-PFC (Fukushima et al. 2004; Hoshi and Tanji 2004), the dorsal premotor cortex (Cisek and Kalaska 2002; Hoshi and Tanji 2000; Ohbayashi et al. 2003; Shen and Alexander 1997), or the posterior parietal cortex (Calton et al. 2002). During the delay period after the first cue appeared, information having both properties was amply represented in the pre-SMA as memory-contingent activity. Even in the late delay period, >30% of pre-SMA neurons selectively represented information given by the first instruction. Sustained memory-contingent activity conveying visual information in the pre-SMA, but not in the SMA, was also reported in a human brain-imaging study (Petit et al. 1998). These results suggest that pre-SMA neurons represent specific instructions, such as arm use or target location, as well as visuospatial properties of the sensory cue and that they retain such information during the delay period for subsequent use.

Implication of pre-SMA activity responding to the second cue

Soon after the second cue appeared, as many as 66% of pre-SMA neurons changed their activity. At this behavioral stage, the fraction of pre-SMA neurons reflecting the information given by the first cue diminished quickly; in parallel, neuronal activity reflecting information provided by the second cue or the combination of information provided by the two cues developed. Remarkably, the information provided by the second cue alone diminished shortly after its appearance. Instead, neuronal activity reflecting information provided by the combination of the two cues became dominant in the pre-SMA (Fig. 11). The combined information signified a component or components of information defining the behavioral properties of a forthcoming action: which arm to use, which target to reach for, or both (Fig. 18). Generating such information seems to require a considerable degree of information processing oriented for behavioral planning. Previously, we reported that neuronal activity exhibiting similar properties, reflecting the information provided by the combination of two cues, was found in the dorsal dl-PFC and PMd of monkeys performing the same behavioral task (Hoshi and Tanji 2000, 2004). It seems probable that neural networks interconnecting the pre-SMA, dorsal dl-PFC, and PMd are involved in such cognitive information processing, previously referred to as “nonstandard sensorimotor integration,” in which an abstract motor instruction is converted into motor commands based on behavioral rules (Mitzi and Wise 1987; Murray et al. 2000; Wise and Murray 2000). This view is consistent with the report showing anatomical connections of the three areas with the PMd as a node (see Fig. 9 in Luppino et al. 2003).
and with reports indicating that the human pre-SMA was active during conditional motor tasks (Kurata et al. 2000; Sakai et al. 1999, 2000).

Matsuzaka and Tanji (1996) reported that a group of pre-SMA neurons exhibited activity particularly when monkeys were changing the direction of a forthcoming reaching movement. More recently, it was reported that a group of pre-SMA neurons exhibited neuronal activity when the subjects were updating sequences of movements (Isoda and Tanji 2004; Shima et al. 1996). In the same vein, Hikosaka and colleagues showed that the pre-SMA was active when subjects were acquiring new sequential procedures (Hikosaka et al. 1996; Nakamura et al. 1998). In a human study using fMRI and repetitive transcranial magnetic stimulation (rTMS) techniques, Rushworth et al. (2002) showed that the pre-SMA was involved in the process of switching visuomotor associations. In contrast, Hernandez et al. (2002) showed that neuronal activity in the pre-SMA and SMA reflects a decision process based on tactile information by identifying three classes of neuronal activity: first- and second-cue signal-related activity and motor-related activity. Deiber et al. (1991) showed that an area corresponding to the pre-SMA was active while subjects were freely selecting the next movements. These reports, along with our findings, suggest that the pre-SMA participates in higher-order aspects of motor control, which range from acquiring or renewing a cognitive or motor set to deciding or selecting the forthcoming motor behavior.

In contrast to pre-SMA neurons, the activity of SMA neurons rarely reflected the information given by the second cue, suggesting that the SMA is not actively involved in the process of motor selection/decision based on visual information. However, the SMA might be involved in motor selection based on somatosensory information. Romo et al. (1993) reported that the SMA was involved in a motor selection process based on the discrimination of vibrotactile signals. This is consistent with anatomical findings that the SMA is connected mainly with the anterior part of the superior parietal lobule and the secondary somatosensory cortex (Luppino et al. 1993), where somatosensory, rather than visual, information is amply represented (Duffy and Burchfiel 1971; Hyvarinen 1982; Jiang et al. 1997; Sakata et al. 1973; Sawamura et al. 2002).

**Implications of SMA and pre-SMA activity during motor preparation and execution**

During the set-cue period before the GO signal appeared, pre-SMA neurons changed their activity more frequently than did SMA neurons. This accords with previous single-cell (Alexander and Crutcher 1990b; Halsband et al. 1994; Matsuzaka et al. 1992) and brain imaging (Cunnington et al. 2002; Jenkins et al. 2000; Lau et al. 2004; see also Yazawa et al. 2000) studies. More importantly, we found that the preparatory activity of pre-SMA neurons preferentially reflected the location of the reach-target, while SMA neurons preferentially reflected arm use, especially in the late preparatory period. Matsuzaka et al. (1992) also found that the spatial location of the target was amply represented in neuronal activity, indicating spatial-target representation in the pre-SMA.

The paucity of arm use selectivity of pre-SMA activity during the preparatory and movement periods supports the view that the pre-SMA exerts its behavioral control in an effector-independent manner. Fujii et al. (2002) showed that, during a peri-movement period, 36% of pre-SMA neurons exhibited similar activity regardless of whether monkeys captured a target with saccadic eye movements or with arm reach. Neuronal activity beyond the confines of the laterality of arm use has also been reported in the PMd (Gentilucci et al. 1988; Hoshi and Tanji 2002; Rizzolatti et al. 1988) and rostral part of the PMd (Cisek et al. 2003), which connect to the pre-SMA. Our preliminary observation also shows that neurons observed in the rostral part of PMd (F2) and the dl-PFC of monkeys performing the current behavioral task reflected target location more than arm use (unpublished observation). Furthermore, it was reported that PMv neurons represent a target in visual space (Kurata and Hoshi 2002; Mushiake et al. 1997) and a visually defined movement trajectory (Schwartz et al. 2004). In area 7 in the inferior parietal lobe, from which the pre-SMA receives direct input, effector-independent (i.e., saccade or arm-movement selective) activity enhancement (Bushnell et al. 1981) and the preference of a world-referenced visual gain field (Snyder et al. 1998) were reported. In addition, during a bilateral arm-reach task, nonlateralized neuronal activity was found more often in area 7 than in area 5 (MacKay 1992; Mountcastle et al. 1975). The combined observations indicate the pre-SMA is involved in the visually based representation of motor targets, rather than in the representation of effectors, via interconnections with cortical areas, including the dl-PFC, PMd, PMv, and area 7 (PG and PFG).

Unexpectedly, in our study, neuronal activity in the pre-SMA during execution of the reaching task was more often suppressed than enhanced (Fig. 2A). This finding is at variance with previous reports from our laboratory (Fujii et al., 2002), in which we found increased activity among pre-SMA neurons. Differences in the behavioral task, including the preparatory process (Crammond and Kalaska 2000), may call for the use of cortical motor areas in a task-specific manner. It is unknown why the selectivity for the target location during the set-cue period was lost during movement execution (Fig. 18) or why neuronal activity was suppressed during this period.

In contrast to the role of pre-SMA neurons, the participation of SMA neurons in movement preparation and execution is well established (Alexander and Crutcher 1990a,b; Crutcher and Alexander 1990; Matsuzaka et al. 1992; Padou-Schioppa et al. 2002, 2004; Romo and Schultz 1992; Tanji and Shima 1994). In our study, we found that a great majority of SMA activity was selective for arm use during either the motor set or peri-movement period. This observation suggests that SMA is involved in specifying the laterality of the arm to be used. However, the selectivity for SMA neurons was much smaller than for muscles (Fig. 19B). Thus the present findings do not imply the role of SMA in specifying muscles or muscle groups to be activated.

It is important to note that SMA neurons exhibited selectivity for use of the ipsilateral and contralateral arm to the same degree during the set period (Fig. 16A), and that 38% of neurons were more active for ipsilateral arm use during movement (Fig. 16C). This means that while performing our motor task, SMA neurons were involved in preparing and executing the reach movement with either arm. How does this observation reconcile with the basically contralateral motor representation of the SMA (Fried et al. 1991; Tanji and Kurata 1979)? Previous studies revealed that, as long as the motor task
required the use of the limb on one side, SMA neurons represented largely contralateral limb movement (Fujii et al. 2002; Tanji and Kurata 1979). When a motor task required the selection of ipsilateral, contralateral, or bilateral digits or arm, the SMA representation was no longer contralateral (Brinkman and Porter 1979; Donchin et al. 2002; Tanji et al. 1987, 1988).

Our findings extend the knowledge of the control of an arm-reach task and indicate that the SMA is involved in specifying the limb to be selected (effector selection) in accordance with task requirements.

Summary and Conclusion

We found that pre-SMA neurons were profoundly involved in 1) receiving visual signals, 2) extracting information about the reach target and arm use, 3) storing necessary information for subsequent use, and 4) combining two sets of information to generate the neuronal activity necessary for making a plan for future reaching movements. These findings suggest that the pre-SMA participates in the early sensorimotor transformation of visual information for behavioral planning. During the motor set period, pre-SMA neurons preferentially represented the location of the reach target, rather than arm use, suggesting that the pre-SMA also participates in the visual guidance of movements, or in representing the target in the extrinsic frame of reference. Combined, our results suggest that the pre-SMA serves as an interface to transform visual information into information required for motor planning, along with the dorso-lateral prefrontal cortex, premotor cortex, and inferior parietal lobule. By contrast, the contribution of the SMA during the early stage of visuomotor transformation appeared meager. Instead, during the preparation and actual execution of reaching movements, information for arm use was preferentially represented in the SMA. Interestingly, however, the activity of SMA neurons was selective for either the ipsilateral or contralateral arm, indicating the participation of the SMA in selecting the use of the right or left arm.

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References


