Covert Representation of Second-Next Movement in the Pre-Supplementary Motor Area of Monkeys

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1Department of Physiology, Tohoku University School of Medicine, Sendai; 2Aihara Complexity Modelling Project, ERATO, JST, Tokyo; and 3Tamagawa University Brain Science Institute, Tokyo, Japan

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Nakajima T, Hosaka R, Mushiake H, Tanji J. Covert representation of second-next movement in the pre-supplementary motor area of monkeys. J Neurophysiol 101: 1883–1889, 2009. First published January 21, 2009; doi:10.1152/jn.90636.2008. We attempted to analyze the nature of premovement activity of neurons in medial motor areas [supplementary motor area (SMA) and pre-SMA] from a perspective of coding multiple movements. Monkeys were trained to perform a series of two movements with an intervening delay: supination or pronation with either forearm. Movements were initially instructed with visual signals but had to be remembered thereafter. Although a well-known type of premovement activity representing the forthcoming movements was found in the two areas, we found an unexpected type of activity that represented a second-next movement before initiating the first of the two movements. Typically in the pre-SMA, such activity selective for the second-next movement peaked before the initiation of the first movement, decayed thereafter, and remained low in magnitude while initiating the second movement. This type of activity may tentatively hold information for the second movement while initiating the first. That information may be fed into another group of neurons that themselves build a preparatory activity required to plan the second movements. Alternatively, the activity could serve as a signal to inhibit a premature exertion of the motor command for the second movement.

INTRODUCTION

One of the essential properties characterizing cortical motor areas is neuronal activity immediately preceding movement onsets that is generally thought to specify movements or represent parameters of movements to be performed (Cheney and Fetz 1980; Crandall and Kalaska 2000; Evarts 1968; Georgopoulos et al. 1982; Tanji and Kurata 1982). Such premovement activity, however, can also be observed in cortical areas outside of motor areas (Kalaska et al. 1983; Mountcastle et al. 1975; Soso and Fetz 1980) and the prefrontal cortex (Quintana and Fuster 1999). Recent studies have reported that the premovement activity in the prefrontal cortex primarily represents behavioral factors that do not belong to motor parameters (Assad et al. 2000; Mushiake et al. 2006; Saito et al. 2005). These reports prompted reconsideration of the nature of the premovement activity. Even in cortical motor areas, the premovement activity may, at least in part, represent aspects of motor behavior separate from specifying immediately impending movements. In asking this question, the pre-supplementary motor area (pre-SMA) is of particular interest because this area has been implicated in behavioral factors other than execution of movements (Clower and Alexander 1998; de Lafuente and Romo 2005; Isoda and Hikosaka 2007; Matsuzaka and Tanji 1996; Shima et al. 1996; Shima and Tanji 2006). With regard to the effect of motor sequence on neuronal activity, the interaction of sequence depth with preferred movement was reported (Sohn and Lee 2007). This finding led us to examine a possibility that pre-SMA neurons could represent the second-next movement in a series of movements, irrespective of immediately impending movement. To address this issue, we trained monkeys to perform two movements, one after the other with an intervening delay, using either the right or left forearm selected appropriately based on memory. We found that neuronal activity in medial cortical motor areas, the pre-SMA in particular, preceding the first movements represents more of the second-next movements than the first movements that are to be immediately initiated. Such activity may constitute a source of input to build a preparatory activity required to plan the second of the two movements while initiating the first.

METHODS

We used two Japanese monkeys (Macaca fuscata, 6.5 and 5.8 kg), cared for in accordance with the Guiding Principles for the Care and Use of Laboratory Animals of the National Institutes of Health. We trained these monkeys to perform a bimanual motor task. During the experiment, each animal sat in a primate chair facing a screen. Eye position was monitored using an infrared eye camera. In each trial of this task, the animals were required to perform a series of two movements. One of four possible movements (left forearm supination or pronation, right forearm supination or pronation) was required for each of the two movements. Therefore the total number of two-movement permutations was 16. For each of the four movements, the deflection angle of arm was 5°. Experimental sessions were sectioned off according to the temporal order of required movements, selected pseudorandomly. Each session consisted of nine trials. The first three trials in a session were performed under visual guidance. The color of the instruction cue; e.g., a red, blue, yellow, or green square, indicated left forearm supination or pronation and right forearm supination or pronation, respectively (Fig. 1A, visually guided trials). In the remaining six trials in the section, the animal performed the movements instructed in the previous visually guided trials without any visual cues (Fig. 1B, memory-guided trials). At the beginning of a trial, the animal was required to place the two manipulanda in neutral positions with its eyes fixated within 5° of the central fixation point (FP) on the screen in front. The monkey had to wait 1.5 s, during which the instruction cue for the first movement was presented for 0.5 s in visually guided trials. Then the central fixation point dimmed; this served as the first movement trigger signal (1st

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The animal was required to perform the first movement within the reaction time limit (1 s), and the manipulandum was returned to the neutral position. While the FP was dimmed, the fixation window was still present: the monkey had to keep fixating on the dimmed FP. After a delay period of 1 s, during which the instruction cue for the second movement was presented for 0.5 s (in visually guided trials), the animal was given the trigger signal for the second movement. A series of correct movements without a fixation break was rewarded with the delivery of juice 500 ms later, followed by a 1.5-s intertrial interval (ITI). During ITI, FP was not presented on the screen, and the eye fixation was not required. After completing six memory-guided trials, a clapping sound signaled the end of the current section and the beginning of a new section.

We used conventional electrophysiological techniques for single-cell recordings. We localized the SMA and pre-SMA based on neuronal responses and the effects of intracortical microstimulation (Matsuzaka et al. 1992). Using electromyography, we recorded the activity of a total of 46 muscles of the forelimb and axis. Although these muscles exhibited movement-related activity, they did not show consistent changes in activity prior to the execution of movements. For the noninstructed arm, we confirmed that there was no muscle activity (in forelimb muscles) nor was there any arm-deflection detectable.

We examined every individual neuron and muscle for all 16 permutations of movements. Typically, 12 memory-guided trials for each series of correct movements were included in the data file. First, we analyzed neuronal activity within the 250-ms period starting 350 ms before the onset of the first movement in the memory-guided trials (PM1 period, Fig. 1B). To estimate the effect of the immediate-next movement and the second-next movement on neuronal activity, we performed a two-way ANOVA ($P < 0.01$). If the immediate-next movement, the second-next movement, or the interaction between the two significantly influenced the neuronal activity during the PM1 period ($P < 0.01$), these neurons were classified as “PM1-neurons.” Within PM1-neurons, neurons with a significant main effect of the second-next movement were determined as “second-next-movement-selective neurons” ($P < 0.01$). Second, the instantaneous firing rate of all PM1-neurons was calculated in a 100-ms time window that was incremented across the trial in 25-ms steps. Then we obtained the sum of the squared deviance of the spike count from its mean (total SS) and the SS for each of the first and second movement (using ANOVA...
tables). The SS value for each movement was divided by the total SS to calculate the selectivity index for each of the first and second movements. The selectivity index was calculated for the data in each time window.

To test whether the number of neurons selective only to the second-next movement was more than expected by chance, we performed a Monte Carlo analysis. For each neuronal data set corresponding to trials including a particular first movement (e.g., right supination), we shuffled the part of neuronal data set corresponding to the second movement. Thereafter, the selectivity of a neuron was re-analyzed by the two-way ANOVA. We repeated this procedure 10,000 times for each recorded neuron and obtained a null distribution of the number of second-next-only-selective neurons in the SMA and pre-SMA. This allowed us to determine the probability that a given number of neurons could be classified as second-next-only-selective by chance, irrespective of the tuning for the immediate-next movement.

**RESULTS**

During single-neuron recordings, the two monkeys performed any permutation of two movements with a success rate of >94% based on memory. We analyzed the activity of 667 PM1-neurons recorded from the SMA and the pre-SMA in the two monkeys. The distribution of neuronal selectivity for each recorded area is shown in Table 1. In this report, typical examples of neuronal activity selective to the immediate-next movement and the activity selective to the second-next movement are illustrated. Subsequent population analysis focuses on the neurons the activity of which was significantly influenced by the second-next movement during the PM1 period ($P < 0.01$).

**IMMEDIATE-MOVEMENT-SELECTIVE ACTIVITY.** Among the 667 PM1 neurons, 40% of SMA and 34% of pre-SMA neurons showed selective activity only for the immediate-next movement during the PM1 period. An example of this type of neuronal activity is shown in Fig. 2A. The activity of this SMA neuron became selectively active during the PM1 period immediately followed by the right arm supination (blue trace in the bottom left panel). This neuron also became active before the right arm supination as the second movement (red trace in the top right panel). Accordingly, the selectivity indices for the first and second movements peaked just before the onset of the corresponding movements (middle panels of Fig. 2A). For this example of neuron, activity broken down into each of the 16 sequences is shown in Fig. 2C.

**SECOND-NEXT-MOVEMENT-SELECTIVE ACTIVITY.** Within PM1-neurons of both areas, we found a group of neurons exhibiting an unexpected type of activity; they showed selectivity for the second movement even before initiation of the first one. An example of this type of neuronal activity is shown in Fig. 2B. The activity recorded in the pre-SMA reflected the second-next movement in an anticipatory manner. It became active only in the trials in which second movements were right arm supination, regardless of the first movements (red trace in the top right panel). A remarkable aspect of the activity is the absence of selectivity for the second movement immediately before execution of the second movement itself (top right panel). The selectivity index for the second movement began to increase >500 ms before the onset of the first movement and peaked during execution of the movement, then decreased rapidly and remained low until the completion of the second movement (middle panels of Fig. 2B). Activity of this example of neuron during performance of each individual sequence is shown in Fig. 2D.

**Population analysis for second-next-movement-selective neurons**

Overall, 81 of 293 (28%) PM1-neurons in the SMA and 136 of 374 (36%) in the pre-SMA were classified as second-next-movement-selective neurons the activity of which was significantly influenced by the second-next movement ($P < 0.01$). Noticeably, neurons selective only to the second-next movement were more prevalent in the pre-SMA (45/374, 12%) than in the SMA (17/293, 6%). The difference in the rate of occurrence of this type of neuron in the two areas was significant at $P = 0.00278$ ($z$ test).

We then analyzed whether the second-next-only-selective neuronal activity occurred more than expected by chance. To test the null hypothesis of occurrences at a chance level, we performed a Monte Carlo analysis. In Fig. 3A, the null distribution is shown of how many neurons in each area could be selective only to the second-next movement by chance when the null hypothesis was true. The actually observed number of second-next-only-selective neurons in the pre-SMA ($n = 45$, shown with a vertical black bar) was significantly more than chance ($P = 0.0031$), whereas that in the SMA ($n = 17$) was not ($P = 0.1012$).

To contrast the properties of second-next-movement selectivity in each area, the instantaneous selectivity index for the second movement was averaged across the neurons whose activity was significantly ($P < 0.001$) influenced by the second-next movement during the PM1 period (SMA: 45 neurons, pre-SMA: 64 neurons). The time course of the selectivity index for the second movement in each area is illustrated in Fig. 3B. Before initiation of the first movement, the mean selectivity index for the second movement became higher in the pre-SMA (yellow trace) than in the SMA, which decreased monotonically thereafter. On the other hand, the selectivity index for the SMA (purple) increased only moderately during the PM1 period, exhibiting a large peak just before the second movement initiation. In Fig. 3C, the time course of the selectivity index for the first movement in each area is shown. For SMA neurons, even though the population was grouped as second-next-movement-selective, the mean selectivity index for the first movement became high and peaked before the initiation of the first movement. In contrast, the index for pre-SMA neurons remained low throughout the analyzed period.

**TABLE 1. Selectivity of neuronal activity during the PM1 period**

<table>
<thead>
<tr>
<th>Selectivity</th>
<th>SMA</th>
<th>Pre-SMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Second-next movement selective” neurons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second-next movement only</td>
<td>17 (6)</td>
<td>45 (12)</td>
</tr>
<tr>
<td>Second-next movement and immediate-next movement</td>
<td>29 (10)</td>
<td>25 (7)</td>
</tr>
<tr>
<td>Second-next movement and interaction*</td>
<td>8 (3)</td>
<td>18 (5)</td>
</tr>
<tr>
<td>Second-next movement, immediate-next movement and interaction*</td>
<td>27 (9)</td>
<td>48 (13)</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate-next movement only</td>
<td>117 (40)</td>
<td>128 (34)</td>
</tr>
<tr>
<td>Interaction* only</td>
<td>30 (10)</td>
<td>60 (16)</td>
</tr>
<tr>
<td>Immediate-next movement and interaction*</td>
<td>65 (22)</td>
<td>50 (13)</td>
</tr>
<tr>
<td>Total</td>
<td>293 (100)</td>
<td>374 (100)</td>
</tr>
</tbody>
</table>

Parentheses enclose percentages. SMA, supplementary motor area. *Interaction between immediate-next movement and 2nd-next movement.
DISCUSSION

In the present study, we analyzed neuronal activity in the SMA and pre-SMA while each monkey was performing a series of two movements based on memory. Although many neurons were found to represent immediate-next movement, the activity of a group of neurons in both areas was significantly influenced by the second-next movement \((P < 0.01)\), even before initiation of the first one. Within the second-nextmovement-selective neurons, neurons exclusively selective to the second-next movement were more prevalent in the pre-SMA than in the SMA. By applying Monte Carlo analysis, we confirmed that the number of second-next-only-selective neurons was significantly more than chance in the pre-SMA but not in the SMA. Moreover, during the premovement period of the first movement, the mean selectivity index for the second movement of pre-SMA neurons was higher than that of SMA neurons. These observations led us to conclude that there certainly was a group of neurons that showed a robust selec-
during a preparatory or premovement period selective to move-
a focus of much interest. In previous studies, neuronal activity (Nakamura et al. 1998, 1999) sequential movements have been

differences in the involvement of these two areas in planning

during properties (Picard and Strick 1996; Tanji 1996). Above all,

separate motor areas with different structural and functional

was more prominent in the pre-SMA.

tivity for the second-next-movement and that the selectivity

appears by chance (calculated with Monte Carlo analysis). Each bar graph indicates the probability that a given number of neurons is classified as “selective only
to the 2nd-next movement.” A vertical line in each panel represents the number of neurons that were actually found to exhibit the particular selectivity. B: time
course of the mean selectivity index for the 2nd movement, averaged across the 2nd-next-movement-selective ($P < 0.001$) neurons in the pre-SMA (yellow trace)

and the SMA (purple trace). Thin black traces indicate standard error. Neuronal activity was first aligned to the onset of the 1st movement (filled triangle) and

the 2nd movement (open triangle), then the selectivity index was calculated successively for each 100-ms time window sliding by steps of 25 ms. Abscissa

represents the time (ms) from the onset of the 1st movement (filled triangle) and the 2nd movement (open triangle). The thick horizontal lines indicate PM1 period

(250 ms). C: time course of the mean selectivity index for the 1st movement with standard error, averaged across the 2nd-next-movement-selective ($P < 0.001$)
nurons in the pre-SMA and SMA. The display format for this figure is the same as for B.

tivity for the second-next-movement and that the selectivity

was more prominent in the pre-SMA.

It has been established that the SMA and pre-SMA are

separate motor areas with different structural and functional

properties (Picard and Strick 1996; Tanji 1996). Above all,

differences in the involvement of these two areas in planning

and executing (Shima and Tanji 1998, 2000) or learning

(Nakamura et al. 1998, 1999) sequential movements have been

a focus of much interest. In previous studies, neuronal activity
during a preparatory or premovement period selective to move-

ment sequences was observed both in the SMA and the

pre-SMA (Mushiake et al. 1991; Shima and Tanji 2000). On

the other hand, neuronal activity reflecting the rank order of

individual movements in a sequence was more commonly

found in the pre-SMA (Clower and Alexander 1998; Shima

and Tanji 2000). In addition, it was reported that activity of

pre-SMA neurons encoded the number of trials in a binary

manner (Shima and Tanji 2006). More recently, Sohn and Lee

(2007) recorded neuronal activity in the pre-SMA and the

SMA while monkeys were performing a series of hand move-

ments under visual guidance. They reported that the number of

remaining movements modulated the intensity of direction-

selective neuronal activity for the immediate-next movement.

Although these results suggest the involvement of SMA and

...
pre-SMA neurons in sequential organization of multiple movements, as far as movement-selectivity is concerned, it remains unclear what the primary variables of each neuron is coding; the sequence, per se, the second-next movement, or the third-next movement. In the present study, we limited the number of movements in a sequence to only two, whereas all permutations of four different movements \((n = 16)\) are included in the analysis. This experimental design allowed us to reveal neuronal activity selective for the second-next movement.

How do the present results relate to previous reports on neuronal activity in other cortical areas? Kettner et al. (1996b) found two categories of activity in the premotor cortex during a delay period preparing to reach two different targets in space: one correlated with the spatial tuning of first movements and the other correlated with tuned responses during second movements. Importantly, in their spatially tuned reaching tasks, premovement activity always coded the direction of forthcoming reaching movements (Kettner et al. 1996a). Thus the premovement activity we report here with our nonspatial motor task is different from previously reported activity. In a more recent study, Batista and Andersen (2001) explored whether a reach-planning activity in the parietal reach region was related to remembering targets for reaches or to specifying the reach-movement to be performed. They found that parietal neurons predominately represented the reach about to be performed and only rarely represented the remembered target for the second reach.

What, then, is the functional implication of the second-next-movement-selective activity in the SMA and pre-SMA? The time course of neuronal activity typically differed between the two areas, as shown in Fig. 3, B and C. For a majority of SMA neurons, the selectivity for the second movement increased gradually until it peaked immediately before the initiation of the second movement. This activity could contribute to the preparation of the second movement, corresponding to a view that the SMA participates in preparing forthcoming movements in general (Alexander and Crutcher 1990; Kurata and Wise 1988; Tanji et al. 1988). In contrast, the selectivity for the second movements in the pre-SMA peaked before initiation of the first movement, decayed thereafter, and remained at a low level at the initiation of the second movement. This time course suggests that it is unlikely that the activity directly specifies motor output for the second movement. One possible interpretation is that such activity may tentatively hold information for the second movement while initiating the first. That information may be fed into another group of neurons that themselves build a preparatory activity required to plan the second movement. Some might raise a possibility that the neuronal activity could be interpreted as holding information about the order of presentation of two visual cues on-line, just like the mnemonic activity in the prefrontal cortex with which the pre-SMA is connected (Picard and Strick 2001). This possibility is unlikely because the second-next-movement-selective activity was generally much greater during performance of memory-guided trials than during visually guided trials and the activity appeared in close time-relation to the occurrence of the first movement (Fig. 2B). Another interpretation of the pre-SMA activity pertains to inhibitory processes. Apart from general inhibition of action (Nachev et al. 2007), recent investigations emphasized the role of the pre-SMA in suppressing unwanted movement (Isoda and Hikosaka 2007; Yang et al. 2008). The second-next-movement-selective activity observed in the present study could serve as a signal to inhibit a premature exertion of the motor command for the second movement. A caveat for this interpretation is that we used a bimanual motor task in the present study. It remains to be studied to what extent the second-next movement selectivity characterizes neuronal activity of medial motor cortex neurons in unimanual motor tasks.

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