

**You might find this additional information useful...**

---

This article cites 21 articles, 9 of which you can access free at:

<http://jn.physiology.org/cgi/content/full/89/5/2868#BIBL>

This article has been cited by 13 other HighWire hosted articles, the first 5 are:

**Role of the Lateral Prefrontal Cortex in Executive Behavioral Control**

J. Tanji and E. Hoshi

*Physiol Rev*, January 1, 2008; 88 (1): 37-57.

[Abstract] [Full Text] [PDF]

**The Representation of Multiple Objects in Prefrontal Neuronal Delay Activity**

M. R. Warden and E. K. Miller

*Cereb Cortex*, September 1, 2007; 17 (suppl\_1): i41-i50.

[Abstract] [Full Text] [PDF]

**Selective involvement of the mid-dorsolateral prefrontal cortex in the coding of the serial order of visual stimuli in working memory**

C. Amiez and M. Petrides

*PNAS*, August 21, 2007; 104 (34): 13786-13791.

[Abstract] [Full Text] [PDF]

**From Numerosity to Ordinal Rank: A Gain-Field Model of Serial Order Representation in Cortical Working Memory**

M. Botvinick and T. Watanabe

*J. Neurosci.*, August 8, 2007; 27 (32): 8636-8642.

[Abstract] [Full Text] [PDF]

**Dynamical Basis of Irregular Spiking in NMDA-Driven Prefrontal Cortex Neurons**

D. Durstewitz and T. Gabriel

*Cereb Cortex*, April 1, 2007; 17 (4): 894-908.

[Abstract] [Full Text] [PDF]

Medline items on this article's topics can be found at <http://highwire.stanford.edu/lists/artbytopic.dtl> on the following topics:

Veterinary Science .. Prefrontal Cortex

Physiology .. Primates

Physiology .. Monkeys

Updated information and services including high-resolution figures, can be found at:

<http://jn.physiology.org/cgi/content/full/89/5/2868>

Additional material and information about *Journal of Neurophysiology* can be found at:

<http://www.the-aps.org/publications/jn>

---

This information is current as of March 7, 2008 .

# Representation of the Temporal Order of Visual Objects in the Primate Lateral Prefrontal Cortex

Yoshihisa Ninokura,<sup>1,2</sup> Hajime Mushiake,<sup>1,2</sup> and Jun Tanji<sup>1,2</sup>

<sup>1</sup>Department of Physiology, Tohoku University School of Medicine, Sendai 980, Japan; <sup>2</sup>The Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Kawaguchi 332-0012, Japan

Submitted 8 August 2002; accepted in final form 6 January 2003

**Ninokura, Yoshihisa, Hajime Mushiake, and Jun Tanji.** Representation of the temporal order of visual objects in the primate lateral prefrontal cortex. *J Neurophysiol* 89: 2868–2873, 2003; 10.1152/jn.00647.2002. Recollecting a past episode involves remembering the temporal order of events. We studied cellular activity in the lateral prefrontal cortex (PFC) of two monkeys that were required to remember the temporal order in which visual objects appeared and to reach for each object in the same order after a delay. Here, we report the cellular activity in the lateral PFC, focusing on the delay period. We found that 43% of the delay-period activity was selective for the sequence in which the visual objects were presented during the cue period. While the majority of cellular activity was selective for multiple sequences, some cells (31%) were selective for only one of six sequences. Our findings show that PFC cells are involved in holding temporal order information when that information is necessary for planning forthcoming motor behavior.

## INTRODUCTION

Remembering the temporal order of events is an important aspect of episodic memory. When temporal-order memory is necessary to organize motor behavior in the future, how and where in the brain is that information represented? The lateral prefrontal cortex (PFC) has been implicated in temporal-order memory. Milner first reported that patients with prefrontal lesions exhibit impaired memory for temporal order (McAndrews and Milner 1991; Milner 1971). The lesion effects were confirmed in later studies (Petrides and Milner 1982; Shimamura et al. 1990; Squire 1982). They were further supported by brain-imaging studies that showed activity foci in the PFC while behavioral tasks were being performed that required the temporal structuring of information that was given to the subject (Cabeza et al. 1997; Petrides et al. 1993). Lesion studies in subhuman primates also revealed the role of the dorsolateral frontal cortex in serial-order memory (Petrides 1991, 1995). However, temporal-order representation has not been studied at the level of cellular activity. Here, we report cellular activity that reflects the temporal order of events presented to the subject, when the order information is essential for planning future motor behavior.

Address for reprint requests: J. Tanji, Department of Physiology, Tohoku University School of Medicine, Sendai 980, Japan (E-mail: tanjij@mail.cc.tohoku.ac.jp).

## METHODS

### Animal and apparatus

We used two male monkeys (*Macaca fuscata*, 6.5 and 5.7 kg) that were cared for according to the National Institutes of Health guidelines and the Guidelines for Animal Care and Use published by our institute. During experimental sessions, the monkeys sat in a chair with their head and left arm restrained and placed their right hand on a touch key in front of the chair. We installed a video monitor equipped with a touch-sensitive screen in front of the monkey, so that it could reach the monitor with its right arm. We used methods, previously described (Hoshi et al. 2000), to monitor and record single-unit activity, electromyographic (EMG) activity, and eye position, and used the TEMPO/Win system (Reflective Computing) to control the behavioral task and to store behavioral and neuronal data for off-line analysis.

### Behavioral task

We trained the monkeys to observe and remember the temporal order in which three visual objects appeared, so that the monkey could plan a subsequent triple-reaching movement in the same order (Fig. 1A). The task started when the animal placed its hand on the touch pad and gazed at a fixation point (FP) that appeared in the center of the monitor, after an intertrial interval of 5 s. If fixation was maintained for 1 s, the monkey was shown three cue objects, each for 0.5 s at 1-s intervals: a yellow circle, a blue rectangle, and a red cross. Since the three objects appeared in a randomized order, the monkey could not use the ordinal number of their appearance as the source of sequence information (cf. Orlov et al. 2000). After a delay of 1.5 s (prechoice delay period), the three objects were displayed together (0.5 s) as a choice cue. The objects appeared to the right, left, or bottom of the FP interchangeably. On the GO signal (disappearance of the fixation point), the animal had to touch the three objects in the order of their appearance during the cue period and was rewarded with fruit juice if it did so in the correct order, with a reaction time < 1 s. While performing the task, we monitored the following muscles bilaterally: the biceps and triceps brachii, deltoid, trapezius, flexor and extensor carpi radialis, supraspinatus, infraspinatus, pectoralis major, rhomboid, and neck and paravertebral muscles. Although the muscles showed movement-related activity, they did not show consistent changes in activity before the actual execution of movements.

### Recording sites

We first identified cortical sulcal patterns and measured the three-dimensional structure around the recording sites using an ultrasound

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

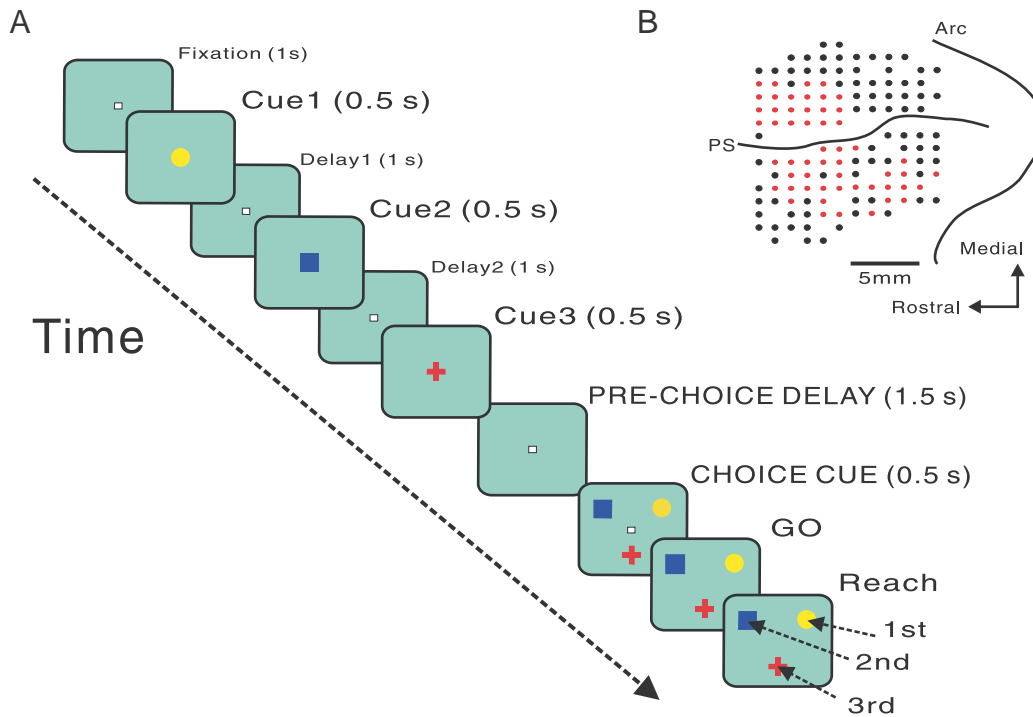


FIG. 1. Behavioral task and recording sites. *A*: temporal sequence of the behavioral events is illustrated schematically from *top left to bottom right*. *B*: cortical recording sites for Monkey 1 are shown in a surface map of the frontal cortex inside the *inset* at the *top right*. Points of electrode entry where task-related prefrontal cortex (PFC) cells were recorded are marked with dots; red dots indicate points where sequence-selective cells were recorded. PS, principal sulcus; Arc, arcuate sulcus.

imaging technique (LOGIQ  $\alpha$  System, GE Medical Systems) (see Tokuno et al. 2000). Subsequently, we applied intracortical microstimulation (ICMS) through the tips of inserted electrodes (11–44 pulses 200- $\mu$ s wide at 333 Hz, current approximately 5–50  $\mu$ A). In this study, we tentatively defined the frontal eye field (FEF) as the area in the anterior bank of the arcuate sulcus where ICMS evoked saccades (with more than 50% probability), with currents < 40  $\mu$ A with 11 pulses. The site that we refer to as the lateral PFC was the portion of the prefrontal cortex rostral to the FEF, corresponding to area 46 of Walker, including both upper and lower banks of the principal sulcus, and a part of areas 9 and 12.

*Data analysis*

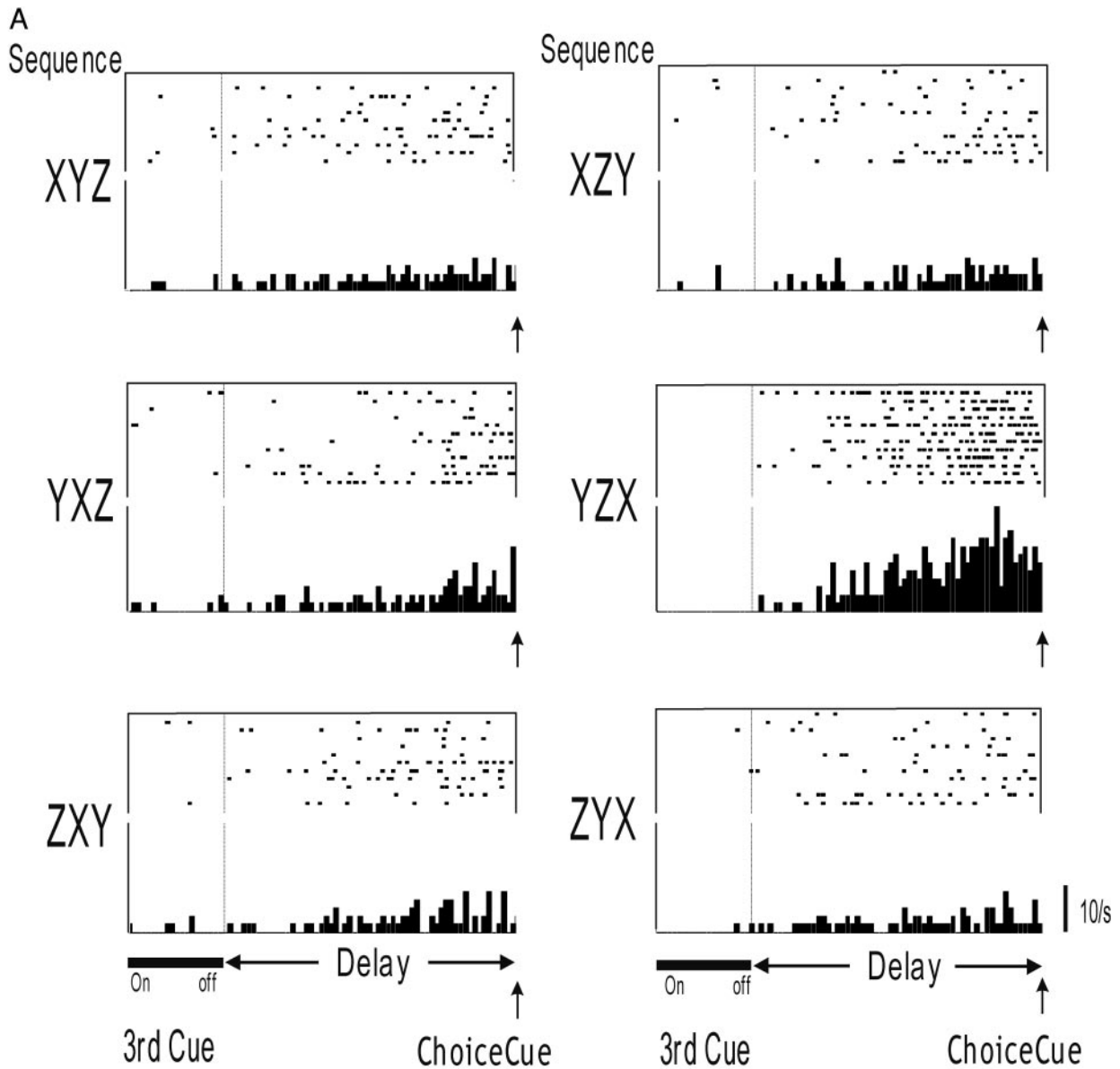
We classified a neuron as “task-related” if its distribution of discharge rates (spikes per second) in the six task periods (prefixation, prefirst cue, sample cue and delay, prechoice delay, choice cue, and movement) was significantly different in at least one of six trial types (Friedman test,  $P < 0.01$ , corrected). This report focuses on neuronal activity in the prechoice delay period, and we will deal with the other task periods in a separate report. For the prechoice delay, we used a time window of 1,000 ms preceding the appearance of the choice cue and determined whether the activity was selective for the sequence of occurrence of the three objects during the sample-cue period. We used ANOVA to examine the relationship between neuronal activity and the six sequences in which the three objects were presented. Where appropriate, individual groups of data (for each sequence) were compared pairwise directly using Tukey’s multiple comparison test.

RESULTS

During single-cell recordings, the two monkeys performed the behavioral task with overall success rates of  $82.6 \pm 6.9$  and  $87.3 \pm 9.2\%$  (mean  $\pm$  SD). We confirmed that the success rate did not depend on the sequence of appearance of the three

objects used for the sample cues ( $P > 0.05$ , ANOVA). Analysis of EMG and eye movement/position data did not detect any sequence-specific postural adjustments or eye movements in either monkey. We found 487 task-related cells in the PFC at the penetration sites shown in Fig. 1 (for Monkey 1). This report focuses on cellular activity in the prechoice delay period. Of the 487 PFC cells, 302 exhibited increased or decreased activity during the prechoice delay period compared with the precue period ( $P < 0.01$ , paired  $t$ -test). Based on the ANOVA, 129 of 302 prechoice delay cells (43%) were found selective for the sequence of appearance of the visual objects ( $P < 0.01$ ). We then performed Tukey’s multiple comparison test to examine for which of the six sequences the delay-period activity was different from others ( $P < 0.01$ ). We found that for 40 of the 129 sequence selective cells (31%), activity changes for only one of the six sequences were significantly different from others. In the example shown in Fig. 2A, the activity was preferentially larger for the sequence YZX (signifying the order blue rectangle, red cross, yellow circle). It is important to note that the spatial location of each object was randomized in the subsequent choice cue. Therefore the selective activity did not reflect expectation for the spatial configuration of the choice cue or the sequence of reaching movements to the three objects. In the 40 one-sequence selective cells, the selectivity was distributed for all of the six sequences, as shown in Fig. 2B.

The remaining 89 sequence-selective cells (69%) were each selective for more than one of the six sequences. Examples of cells exhibiting selectivity for two, three, and four sequences are shown in Fig. 3: cell 1 was selective for YXZ and YZX; cell 2 for XZY, YZX, and ZYX; and cell 3 for XYZ, XZY, YXZ, and YZX. The distribution of selectivity for the 94 cells



**B** Distribution of One-sequence Selectivity



FIG. 2. A: activity of a PFC cell exhibiting selectivity to the appearance of the sample cues in the order YZX. In the raster displays, each row represents a trial and each dot shows when the cell discharged. Discharges during the prechoice delay period are aligned at the onset of the choice cue. Below each raster display, perievent histograms are drawn with 40-ms bins. B: distribution of PFC cells whose prechoice delay activity was selective for 1 of the 6 cue sequences.

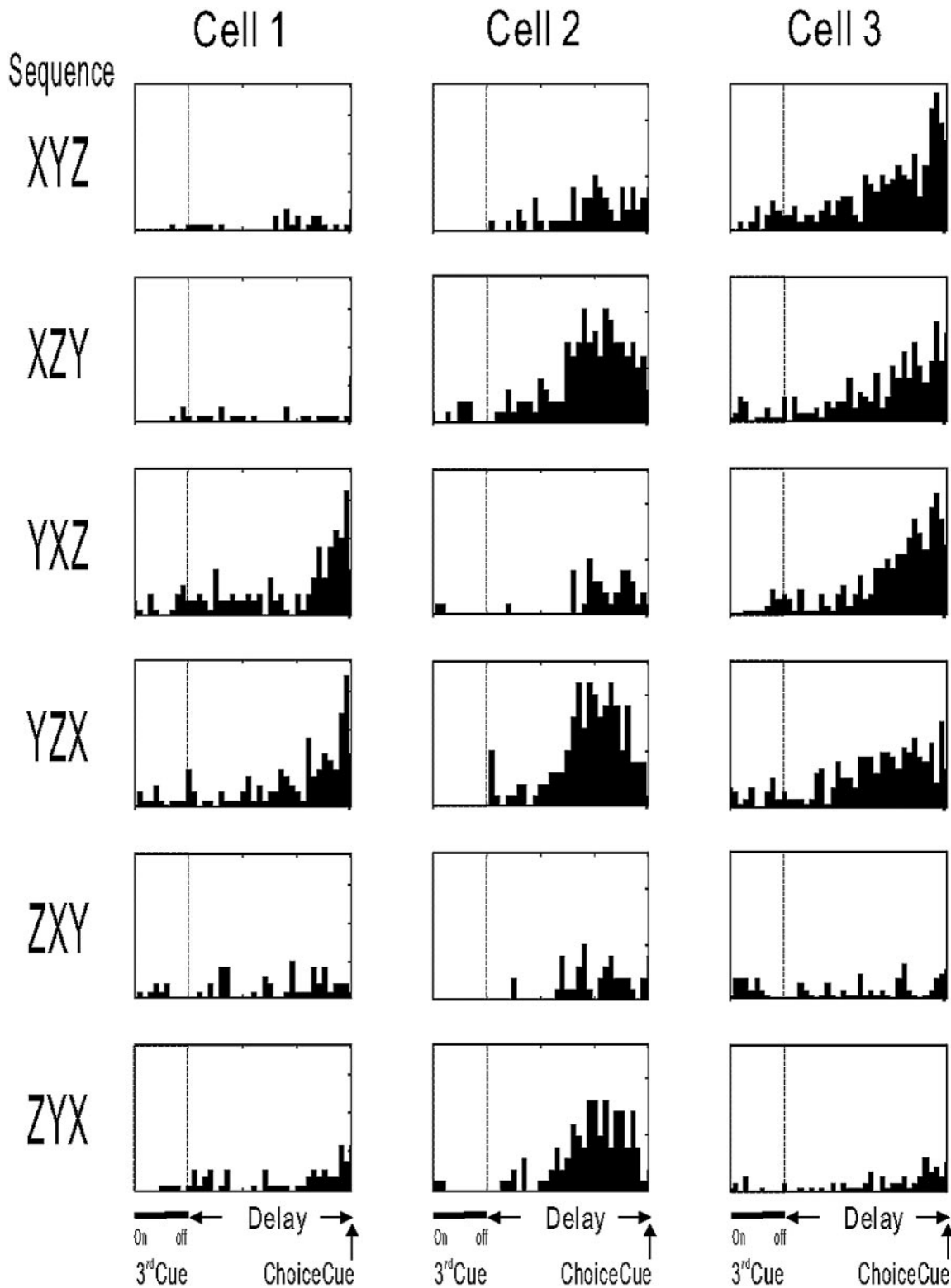


FIG. 3. Examples of PFC cells whose delay activity was selective for 2 (left), 3 (middle), or 4 (right) of the 6 sequences. One step in the ordinate denotes 20 spikes/s.

to two, three, four, or five of the six sequences is shown in Table 1. We investigated whether characteristic temporal structures were involved in the sequence to which the 89 cells were selectively active. First, we counted the number of cells that were selective to sequences in which a particular object (such

as X) appeared at the first, second, or third positions among the three objects. For instance, cell 1 shown in Fig. 3 was selectively active when object Y appeared first in the sequence. However, the overall occurrence of such putative numerical-position selectivity did not exceed the theoretical probability of

TABLE 1. Distribution of the sequence-selectivity of lateral PFC cells during the delay period

Number of Sequences for Which the Cells Were Selective	Number of Cells		
	Monkey 1	Monkey 2	Total
1	32	8	40
2	21	13	34
3	12	3	15
4	19	8	27
5	10	3	13
Nonsselective	125	48	173
Total	219	83	302

appearance (of a particular object at 1st, 2nd, or 3rd numerical positions) in all of the sequences ( $P > 0.05$ ,  $\chi^2$  test). We then examined whether the order of any two objects (e.g., X followed by Y) was a significant factor contributing to the selectivity. Such "chunking" of two-object sequences did not activate more cells than was expected randomly ( $P > 0.05$ ,  $\chi^2$  test).

Furthermore, the activity of 173 cells during the prechoice delay period was not selective to the sequence. The locations of the recording sites of the sequence-selective and nonsselective cells are shown in Fig. 1B. Sequence-selective cells were distributed in the central part of the lateral PFC, including the banks of the principal sulcus, and extended into both the dorsal and ventral parts of the periprincipal areas.

## DISCUSSION

In this study, we found that cellular activity in the lateral PFC exhibited selectivity to the temporal order of the visual occurrence of objects when the sequence information was important for planning a subsequent motor task. For some cells, the activity was selective for only one of the six sequences, while for others the selectivity was for two, three, four, or five of the six sequences. Since the success rates in achieving the task did not depend on the sequence, the selective activity could not be explained by behavioral variables such as task difficulty, attentiveness to the task, or general arousal. In this behavioral task, the monkey was required to detect three visual objects and to retain the order of their appearance in memory during the prechoice delay period, to prepare for a motor task that involved reaching for the three objects in the same order. The selective activity of prefrontal cells was observed during this period of short-term retention of order information, in preparation for using the information. It is important to note that the monkey had no knowledge of the spatial configuration of the three choice cues during the delay period. Therefore the neuronal activity was not related to planning or preparing actual motor processes that involved reaching for the three spatial targets.

In the present behavioral task, it is theoretically possible for the monkeys to remember the order of appearance of the first two objects and to assign the remaining object to the third temporal position. Although this possibility could not be ruled out, the following two findings made it more likely that the monkeys used the three cues to solve the task. First, in the delay period after the second cue (delay 2), neuronal activity rarely reflected the sequence of cue 1 and cue 2. Instead, the delay 2 activity reflected the second cue only. In most cases, the sequence-selective delay activity did not appear before the

prechoice delay period. Second, responses to the third cue were as frequent as responses to the first and second cues, indicating equal attentiveness to the three cues. We wish to report neuronal activity during delay 1 and delay 2, as well as cue responses, in a separate report.

It is well known that damage to the human frontal cortex can cause severe deficits in certain aspects of mnemonic performance. These deficits include impairment in tasks requiring the active monitoring of a series of self-ordered responses (Petrides and Milner 1982) and the recall of the temporal order of stimuli (Milner 1971; Milner et al. 1991; Shimamura et al. 1990). In subhuman primates, Petrides (1995) reported that lesions of the dorsolateral frontal cortex impaired memory for the order of a series of visual stimuli. That study extended his previous observations of the impairment of those motor tasks that required the monitoring of the temporal order of self-action (Petrides 1991) and provided a basis for the view that the dorsolateral PFC is crucially involved in monitoring and manipulating information about the order of events. Our findings support this view and demonstrate how the activity of cells in the lateral PFC take part in representing the order of nonspatial visual information. The cellular activity selective for only one of the six sequences that we found is appropriate for encoding a particular order of appearance of visual objects. On the other hand, cells selective for more than one sequence may participate in encoding the temporal order as a population.

The PFC has been implicated in a variety of cognitive processes, including the short-term storage of visual (Fuster and Alexander 1971; Goldman-Rakic 1987; Miller et al. 1996) and somatosensory (Romo et al. 1999) information. Recent studies have revealed the retention of information at more abstract levels, including rule-dependent (Wallis et al. 2001; White and Wise 1999), task-selective (Asaad et al. 2000; Hoshi et al. 1998), and category-specific (Freedman et al. 2001) activity. Concerning the serial-order information, a previous report described PFC activity selective for spatiotemporal patterns of the appearance of visual targets for subsequent sequential saccades (Barone and Joseph 1989). For instance, in that study, a PFC cell was active selectively when three spatial targets appeared in the order up, left, and right, relative to a fixation point. Such activity could have reflected a particular order of spatial targets or the preparation of three saccades in that order and was interpreted as useful for constructing oculomotor plans to perform multiple saccades with a specific spatiotemporal pattern. The order information that we discovered has implications that differ from those of the previous report in two respects. First, the order-selective information is formulated at a stage before the actual motor behavior is planned, although the information may, in part, reflect the formation of a motor plan at an abstract level (e.g., a plan to reach to a red, blue, and yellow object in that order). Second, the order information is not confounded by spatial-order information, emphasizing the temporal pattern in the occurrence of visual objects. Taken together, our results suggest the manner in which prefrontal cells take part in encoding episodic memory on the order of visual events.

We thank M. Kurama and Y. Takahashi for technical assistance. This work was supported by Japan Science and Technology Corporation.

## REFERENCES

- Asaad WF, Rainer G, and Miller EK.** Task-specific neural activity in the primate prefrontal cortex. *J Neurophysiol* 84: 451–459, 2000.
- Barone P and Joseph J-P.** Prefrontal cortex and spatial sequencing in macaque monkey. *Exp Brain Res* 78: 447–464, 1989.
- Cabeza R, Mangels J, Nyberg L, Habib R, Houle S, McIntosh AR, and Tulving E.** Brain regions differentially involved in remembering what and when: a PET study. *Neuron* 19: 863–870, 1997.
- Freedman DJ, Riesenhuber M, Poggio T, and Miller EK.** Categorization of visual stimuli in the primate prefrontal cortex. *Science* 291: 312–316, 2001.
- Fuster JM and Alexander GE.** Neuron activity related to short-term memory. *Science* 173: 652–654, 1971.
- Goldman-Rakic PS.** Circuitry of primate prefrontal cortex and regulation of behavior by representational memory. In: *Handbook of Physiology. The Nervous System. Higher Functions of the Brain*. Bethesda, MD: Am. Physiol. Soc., 1987, sect. 1, vol. V, p. 313–417.
- Hoshi E, Shima K, and Tanji J.** Task-dependent selectivity of movement-related neuronal activity in the primate prefrontal cortex. *J Neurophysiol* 80: 3392–3397, 1998.
- Hoshi E, Shima K, and Tanji J.** Neuronal activity in the primate prefrontal cortex in the process of motor selection based on two behavioral rules. *J Neurophysiol* 83: 2355–2373, 2000.
- McAndrews MP and Milner B.** The frontal cortex and memory for temporal order. *Neuropsychologia* 29: 849–859, 1991.
- Miller EK, Erickson C, and Desimone R.** Neural mechanisms of visual working memory in prefrontal cortex of the macaque. *J Neurosci* 16: 5154–5167, 1996.
- Milner B.** Interhemispheric differences in the localization of psychological processes in man. *Br Med Bull* 27: 272–277, 1971.
- Milner B, Corsi P, and Leonard G.** Frontal-lobe contribution to recency judgments. *Neuropsychologia* 29: 601–618, 1991.
- Orlov T, Yakovlev V, Hochstein S, and Zohary E.** Macaque monkeys categorize images by their ordinal number. *Nature* 404: 77–80, 2000.
- Petrides M.** Functional specialization within the dorsolateral frontal cortex for serial order memory. *Proc R Soc Lond B* 246: 299–306, 1991.
- Petrides M.** Impairments on nonspatial self-ordered and externally ordered working memory tasks after lesions of the mid-dorsal part of the lateral frontal cortex in the monkey. *J Neurosci* 15: 359–375, 1995.
- Petrides M, Alivisatos B, Meyer E, and Evans AC.** Functional activation of the human frontal cortex during the performance of verbal working memory tasks. *Proc Natl Acad Sci USA* 90: 878–882, 1993.
- Petrides M and Milner B.** Deficits on subject-ordered tasks after frontal- and temporal-lobe lesions in man. *Neuropsychologia* 20: 249–262, 1982.
- Romo R, Brody CD, Hernandez A, and Lemus L.** Neuronal correlates of parametric working memory in the prefrontal cortex. *Nature* 399: 470–472, 1999.
- Shimamura AP, Janowsky JS, and Squire LR.** Memory for the temporal order of events in patients with frontal lobe lesions and amnesic patients. *Neuropsychologia* 28: 803–813, 1990.
- Squire LR.** Comparisons between forms of amnesia: some deficits are unique to Korsakoff's syndrome. *J Exp Psychol* 8: 560–571, 1982.
- Tokuno H, Hatanaka N, Takada M, and Nambu A.** B-mode and color Doppler ultrasound imaging for localization of microelectrode in monkey brain. *Neurosci Res* 36: 335–338, 2000.
- Wallis JD, Anderson KC, and Miller EK.** Single neurons in prefrontal cortex encode abstract rules. *Nature* 408: 466–470, 2001.
- White IM and Wise SP.** Rule-dependent neuronal activity in the prefrontal cortex. *Exp Brain Res* 126: 315–335, 1999.