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Integration of Temporal Order and Object Information in the Monkey Lateral Prefrontal Cortex

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Ninokura, Yoshihisa, Hajime Mushiake, and Jun Tanji. Integration of temporal order and object information in the monkey lateral prefrontal cortex. *J Neurophysiol* 91: 555–560, 2004. First published September 10, 2003; 10.1152/jn.00694.2003. Generation of information about the temporal order of events is essential for the control of memory-based behavioral tasks. We studied cellular activity in the lateral prefrontal cortex (LPFC) in two monkeys that were required to remember the temporal order in which visual objects were presented. In this report, we focus on cellular activity in response to the sequential appearance of three different objects. We identified cells that responded selectively to physical properties (color and shape) of objects (23%) in the ventral part of the LPFC and cells for which activity was selective for the numerical position (rank order) of objects (44%) in the dorsal part of the LPFC. We also identified cells for which activity was selective for both the physical properties and rank order of objects (30%). The third type of cells, distributed in the ventral LPFC, seems of importance in integrating the two categories of information, i.e., physical and temporal information about the occurrences of objects to construct sequential order information. Furthermore, we identified a distinct group of cells that exhibited selectivity for the sequence of presentation of the three objects. Our findings suggest that LPFC cells are involved in encoding temporal sequences of events when such information is required for planning forthcoming motor behavior.

INTRODUCTION

Storing information about the temporal sequence of events for future use is one of the fundamental processes in which the lateral prefrontal cortex (LPFC) is involved (Cabeza et al. 1997; Milner 1971; Petrides 1991; Petrides et al. 1993; Shimamura et al. 1990; Squire 1982). In a recent report from our laboratory, we presented evidence that cellular activity in the LPFC reflects the temporal order of visual objects presented to a subject in the process of planning future actions based on memorized information about the temporal order of presentation (Ninokura et al. 2003). These observations led to the question of how event-sequence information is encoded by neural elements.

To generate information about the sequence of events, it is necessary to detect the physical attributes of individual events and arrange individual events in the temporal order in which they appear. In this study, we investigated how the sensory information that characterizes events and the temporal information about event appearance are reflected in the activity of individual neurons in the LPFC of monkeys that were required to determine the sequential order in which several objects were

presented. We demonstrated that both the physical attributes of objects and the order of their appearance (rank within a sequence) are reflected in neuronal activity within the LPFC, either individually or together.

METHODS

Animals and apparatus

We used two male monkeys (*Macaca fuscata*) that had been used in a previous study (Ninokura et al. 2003). They were cared for according to National Institutes of Health guidelines and the Guidelines for Animal Care and Use published by our institute. During experimental sessions, the monkeys were seated in a primate chair with their head and left arm restrained and could place their right hand on a touch pad that was positioned in front of the chair. A video monitor equipped with a touch-sensitive screen was placed in front of the monkey such that it could reach the monitor with its right arm. We used the methods described by Hoshi et al. (2000) to monitor and record single-unit activity in the LPFC, electromyographic (EMG) activity, and eye position. We used the TEMPO/Win system (Reflective Computing, St. Louis, MO) to control the behavioral task and to store behavioral and neuronal data for off-line analysis.

Behavioral task

The monkeys were trained 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 one of three cue objects (a yellow circle, a blue rectangle, or a red cross, each 2° in size) for 0.5 s each at 1-s intervals. The objects were presented in a randomized order. Therefore the monkey could not use the ordinal number of the appearance of each object as a source of sequence information (Orlov et al. 2000). After a delay of 1.5 s (prechoice delay period), the three objects were displayed simultaneously for 0.5 s, which served as a choice cue. The objects appeared interchangeably to the right, left, or bottom of the FP. On disappearance of the FP (go signal), the animal was required to touch the three objects in the same order in which they had appeared during the cue period. The animal was rewarded with fruit juice if it correctly replicated the order of the objects with a reaction time <1 s. While performing the task, we monitored the following muscles bilaterally: biceps and triceps brachii, deltoid, trapezius, flexor and extensor carpi radialis, supraspinatus, infraspinatus, pectoralis major, rhomboid, and neck and paravertebral muscles. Although

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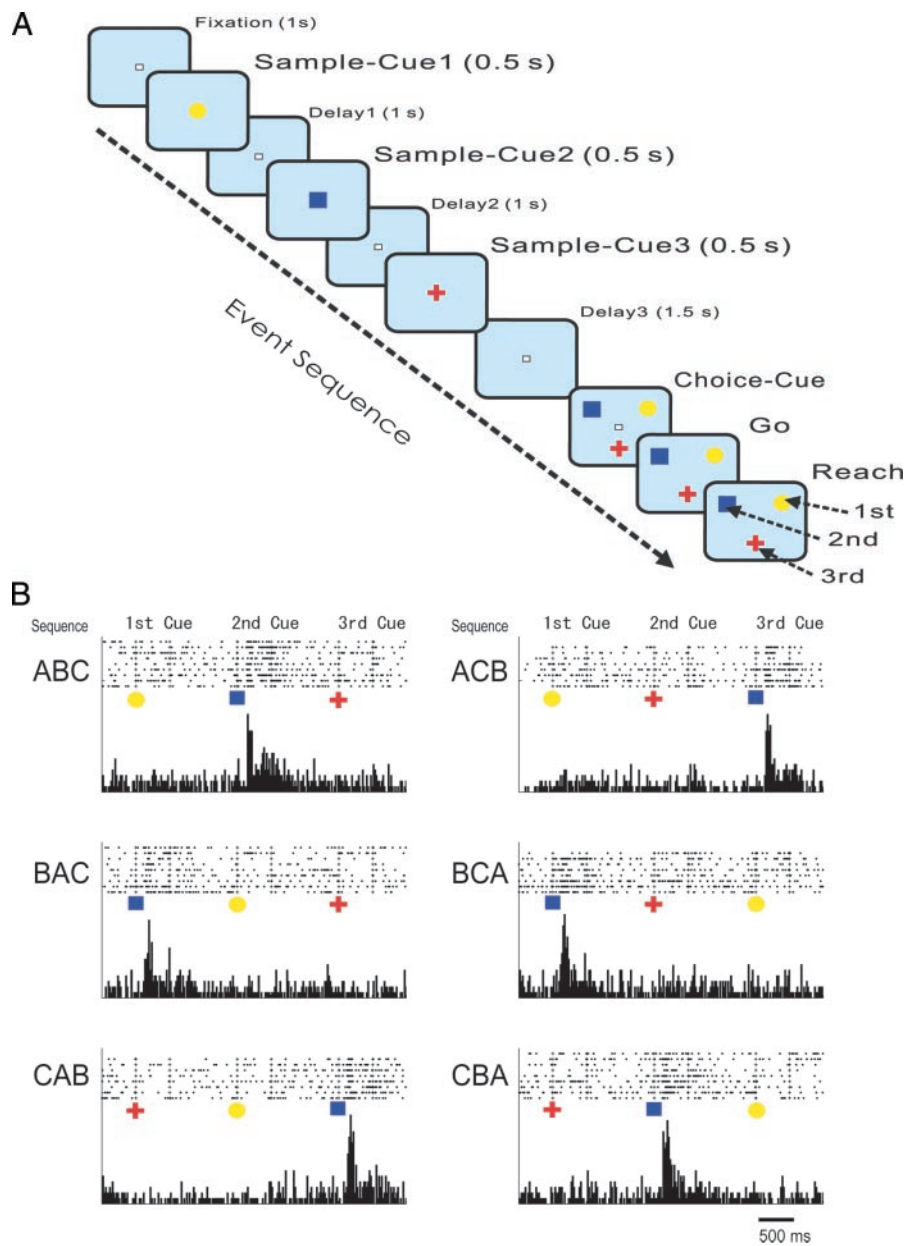


FIG. 1. Behavioral task and object-selective cellular activity. *A*: temporal sequence of behavioral events illustrated schematically from top left to bottom right. *B*: activity of a representative lateral prefrontal cortex (LPFC) cell that exhibited selective responses to the appearance of a sample object (cue B, blue rectangle). In the raster displays, each row represents a trial and each dot shows when the cell discharged. Discharges during the sample-cue period are aligned with the onset of the choice cue. The onset and offset of each sample cue are denoted with dotted lines. The peri-event histograms shown below each raster display were constructed using 40 ms bins.

the muscles exhibited movement-related activity, they did not show consistent changes in activity prior to the execution of movements.

Recording sites

We first identified cortical sulcal patterns and measured the three-dimensional structure around the recording sites using ultrasound imaging (LOGIQ α System, GE Medical Systems, Milwaukee, WI) (see Tokuno et al. 2000). Subsequently, we applied intracortical microstimulation through the tips of microelectrodes (11 ~ 44 pulses of 200 μ s at 333 Hz, current: 5 ~ 50 μ A). In this study, we tentatively defined the frontal eye field as the area in the anterior bank of the arcuate sulcus in which microstimulation (current <40 μ A, 11 pulses) evoked saccades at a probability >50%. After recording neural activity, the monkeys were killed with pentobarbital sodium and perfused transcardially with saline followed by 8% formaldehyde with 3% potassium ferrocyanide. Serial coronal brain sections 50 μ m thick were cut and Nissl-stained. The tracks of the recording electrodes were reconstructed using iron deposits as reference points, which were

formed at the electrode tips by passing a positive DC current through the electrode. The site that we refer to as the lateral PFC was the portion of the prefrontal cortex rostral to the frontal eye field, which corresponds to area 46 of Walker and includes both the upper and lower banks of the principal sulcus and a portion of areas 9 and 12.

Data analysis

We classified a neuron as task related if the distribution of its discharge rates (spikes/second) in the six task periods (fixation or prefirst cue, sample cue and delay, prechoice delay, choice cue, and movement) differed significantly (Friedman test, $P < 0.01$, corrected), during performance of at least one of six types of trials in which objects appeared with six different sequences. This report focuses on neuronal activity during the three sample-cue periods. (We will address the remaining task periods in subsequent reports.) For the sample-cue periods, we selected a window of 500 ms after appearance of the choice cue and determined whether neuronal activity was selective for the physical properties (color and shape) or numerical

position (rank order) of the visual objects. We used two-way ANOVA ($P < 0.01$) to examine the relationships between neuronal activity, the physical properties of the cues, and the rank order of the cues. Where appropriate, individual groups of data (for each sequence) were compared pairwise directly using Tukey's test ($P < 0.01$).

RESULTS

During single-cell recordings, the two monkeys performed the behavioral task with a success rate of 83.7 ± 7.1 and $88.2 \pm 9.1\%$ (means \pm SD), respectively. The success rate did not depend on the sequence of appearance of the sample cues ($P > 0.05$, ANOVA). No sequence-specific postural adjustments or eye movements were apparent in the EMG or eye-movement/position data for either monkey.

We identified 932 task-related cells in the LPFC at the recording sites shown in Fig. 3. Of these cells, 539 exhibited increased or decreased activity during the sample-cue periods compared with the precue period (paired t -test, $P < 0.01$). We found that 141 of the 539 sample-cue related cells (26%) were selective for the physical properties of the cues. Most of them

(96%) exhibited selective activity increase, while only a minority exhibited decrease of activity. An example of such object selectivity is shown in Fig. 1B, in which a LPFC cell can be seen to respond preferentially to the blue rectangle. In contrast, the activity of 235 LPFC cells (44%) was selective for rank, irrespective of the physical properties of objects. Most of them (94%) exhibited selective activity increase. Two examples of such rank selectivity are shown in Fig. 2, in which the activity of *cell 1* is selective for the first cue, whereas that of *cell 2* is selective for the second cue. Among the 235 rank-selective cells, 94, 63, and 39 cells were selective for the first, second, and third rank, respectively. The remaining 39 cells were selective for more than one of the three ranks; 18, 11, and 10 cells were selective for the ranks 1–2, 2–3, and 2–3, respectively. We found that 163 cells (30% of 539) were selective for both the physical properties of objects as well as their rank. An example is shown in Fig. 2, *right*, in which a LPFC cell (*cell 3*) responded exclusively to one of the three objects (yellow circle) when this object was presented as the second cue but not when the same object was presented as the first or third cue.

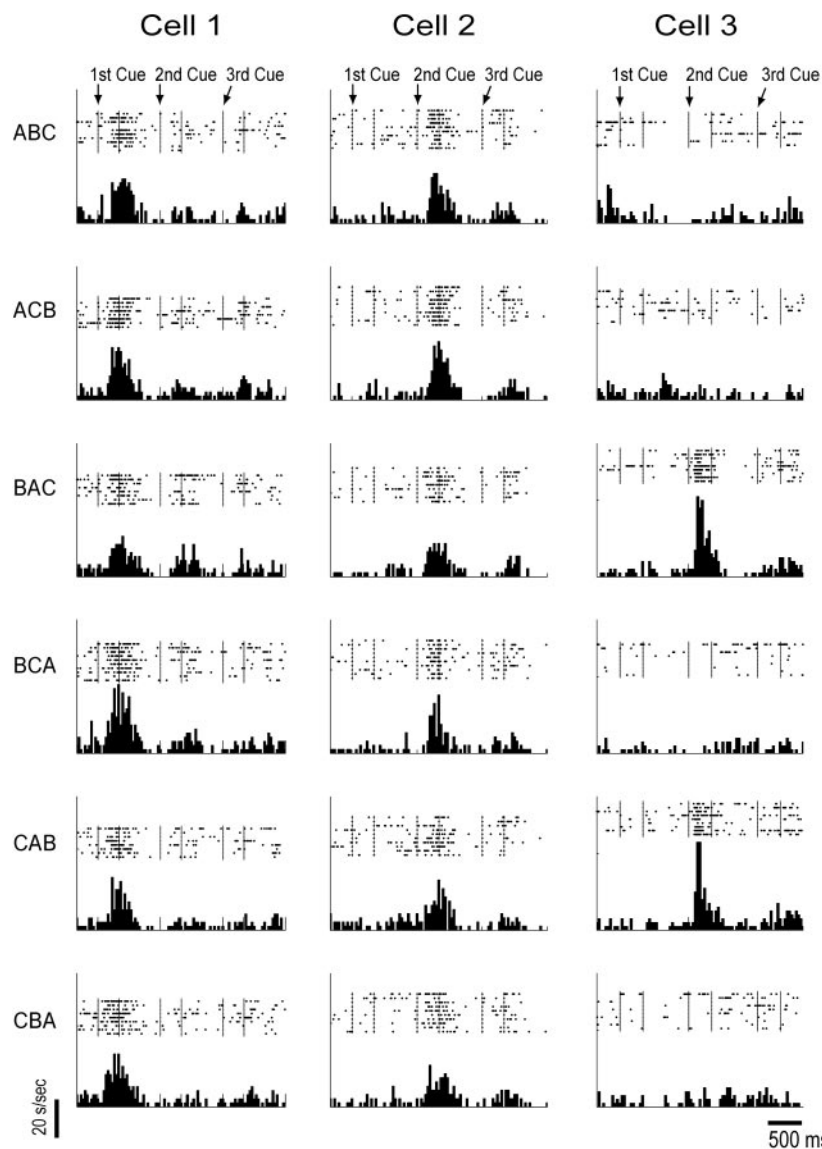


FIG. 2. Three examples of LPFC cells that exhibited rank-order selectivity in cue responses. *Cell 1* was active selectively in response to the 1st cues, whereas *cell 2* was active selectively in response to the 2nd cues irrespective of the physical properties (shape and color) of the cues. *Cell 3* responded to the 2nd cue only if the cue was A (yellow circle). Display formats are the same as in Fig. 1B.

Selectivity for physical properties and rank was found in 69, 55, and 39 cells in response to the first, second, and third cue, respectively.

We subsequently examined the possibility that the sample cue response is selective for a particular sequence of object presentation. As the temporal sequence of object presentation was randomized, monkeys could not predict which of the three objects would appear first. Therefore responses to the first presentation of a cue in each sequence were excluded from the analysis. As described in the preceding text, responses to the sample cue were often selective for object or rank. Therefore we were able to determine the sample cue responses as being sequence selective only if responses to a particular object that appeared at a specific rank differed according to the temporal order of appearance of each object. For these reasons, we analyzed whether one of the three objects appearing in either the second or third rank gave rise to statistically different responses ($P < 0.01$, Mann-Whitney U test) depending on the sequence of object appearance. Two examples of sequence-selective activity are shown in Fig. 3A, in which *cell 1* ap-

peared to be active in response to cue B (blue square) if the preceding cue was a yellow circle and the following cue was a red cross. As shown in Fig. 3A, *cell 2* responded to a red cross if the sequence of objects was blue square-yellow circle-red cross. We found that 22 LPFC cells exhibited sequence selectivity for the response in the second rank, whereas another 22 cells appeared to be sequence-selective for the third rank.

The locations of the recording sites in which object- and rank-selective cells were identified are shown in Fig. 3B. Object-selective cells were distributed ventral to the principal sulcus, whereas rank-selective cells were located in the dorsal bank of the principal sulcus and in the cortical surface between the principal sulcus and the upper limb of the arcuate sulcus. The distribution of cells that exhibited both object and rank selectivity overlapped extensively with the distribution of object-selective cells. We noticed some tendency for clustering of similar types of responses when more than one single unit was recorded at a given electrode, but the number of two-cell recordings were not sufficiently large to try any statistics.

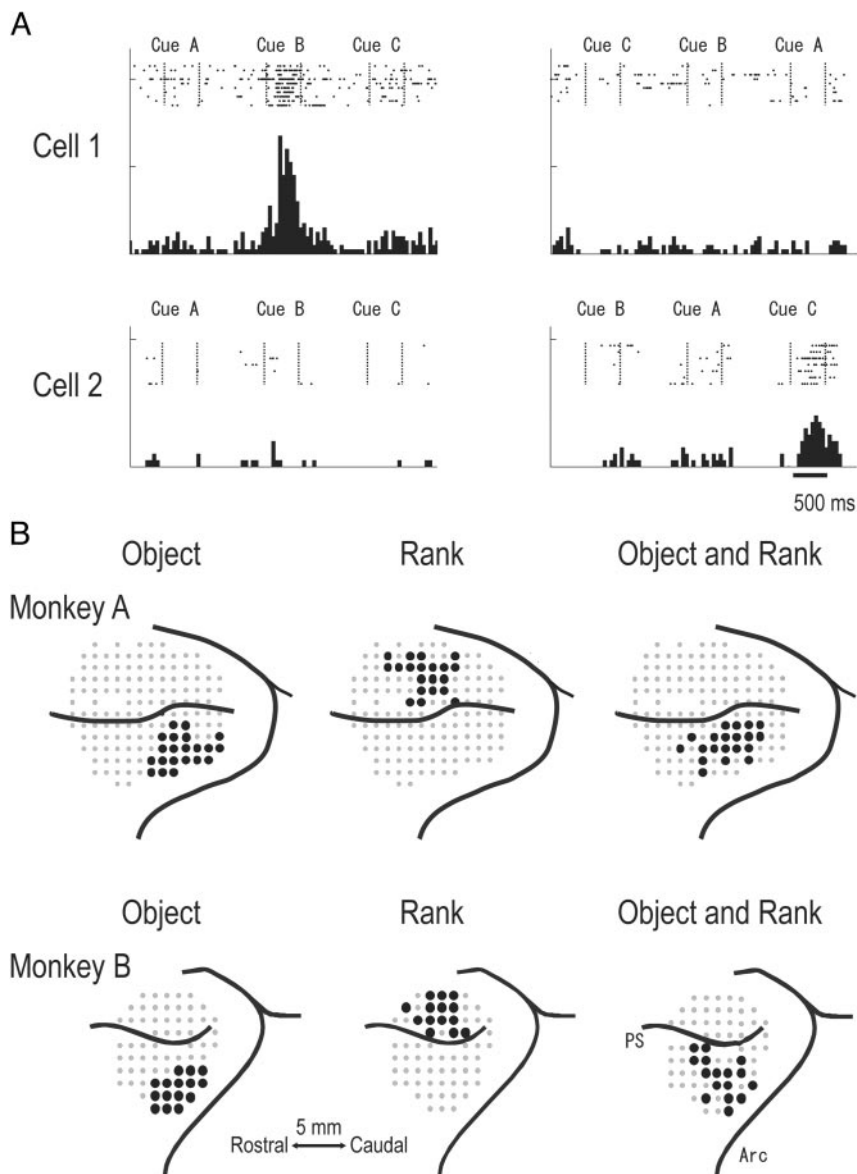


FIG. 3. Sequence-selective LPFC activity and recording sites. A: discharges of LPFC cells that exhibited selective activity for the cue sequence A-B-C (*cell 1*) or B-A-C (*cell 2*). B: cortical recording sites for *monkeys A* and *B* shown as surface maps of the frontal cortex rostral to the arcuate sulcus. The locations of electrodes that recorded task-related LPFC activity are marked with dots. Filled circles indicate points at which object-selective, rank-selective, object- and rank-selective cellular activity was recorded. PS, principal sulcus; Arc, arcuate sulcus.

DISCUSSION

In the present study, we showed that the majority of neuronal activity in LPFC cells in response to visual objects was influenced by the rank of presentation of the object (rank order selectivity). Forty-four percent of cells were selective to rank order irrespective of the physical properties of objects, whereas 30% of cells were selective to both the rank order and physical properties of objects. In addition, we identified cells that responded selectively to one of three visual objects if it appeared at a particular time in the presentation sequence.

In the behavioral task used in the present study, monkeys were required to reach and press three cues in the same order in which they had been presented during a sample cue period. To perform this task, the monkey had to generate information regarding the temporal order of appearance of the three cues prior to preparing to initiate reaching in the correct order. To construct sequential order information, it was necessary to detect the physical properties of each of the three cue objects, register the order of appearance (rank) of individual objects, and integrate the two categories of information, i.e., physical and temporal information. The existence of three types of cellular activity (i.e., object, rank, and combination selective) suggests that LPFC cells are involved in encoding of the temporal order information. These cells, together with those that respond to an object in a sequence-selective manner, appear to be useful for relaying information to cells that are active during the prechoice delay period to generate sequence-selective activity during short-term retention of order information (Ninokura et al. 2003). We found that all types of cellular responses described in this study were attenuated when monkeys made errors, although infrequent occurrences of errors per particular types of responses precluded statistical analysis of error effects.

Milner (1971) was the first to report that patients with damage in the frontal cortex develop disturbances in the temporal ordering of events. Subsequently, in more refined studies, patients with frontal lobe lesions were shown to be impaired in tasks that required active monitoring of a series of self-ordered responses (Petrides and Milner 1982) and in recalling the temporal order of stimuli (Shimamura et al. 1990; Milner et al. 1991). The involvement of the LPFC in the temporal processing of memorized information has also been inferred by brain imaging studies, which revealed that there are foci of activity in the LPFC during behavioral tasks that require the temporal structuring of visual information (Cabeza et al. 1997; Petrides et al. 1993). Petrides (1995) reported that lesions of the dorsolateral frontal cortex in non-human primates impaired memorization of the order of a series of visual stimuli, thereby inferring that the dorsolateral prefrontal cortex is crucially involved in monitoring and manipulating information about the order of events. The findings presented here support these previous studies and provide novel insights into how the activity of individual LPFC cells is related to the encoding of the temporal order in which objects appear.

Recent studies have revealed that the responses of cells in the LPFC to visual signals are influenced profoundly by various factors of behavioral significance, including behavioral rules (Wallis et al. 2001; White and Wise 1999), task requirements (Asaad et al. 2000; Hoshi et al. 1998; Sakagami and Niki 1994), quantity detection (Nieder et al. 2002), and category

specification (Freedman et al. 2001, 2003). The present study suggests that an additional behavioral factor might influence the visual responses of LPFC cells, namely the temporal order of information. In a previous study, Barone and Joseph (1989) reported that LPFC activity was selective for the spatiotemporal pattern of the appearance of visual targets in subsequent sequential saccades. They studied activity of LPFC cells in response to three spatial targets presented serially above, to the left, and to the right of the FP and found that spatially selective responses were influenced by the temporal or spatial sequence in which the targets were presented. In this early study, selective responses of cells to the rank of the three cues were not described. However, rank-selective activity was described subsequently in the caudate nucleus (Kermadi and Joseph 1995), supplementary and presupplementary motor areas (Clower and Alexander 1998; Shima and Tanji 2000), and in the cingulate motor area (Procyk et al. 2000). In the present study, we found that the numerical order of cues is used by cells in the LPFC to construct a memory of temporal structure for several objects, thus providing an experimental model in which LPFC cells are involved in encoding episodic memory.

We found that object-feature-selective cells were localized to the lateral PFC, ventral to the principal sulcus. This is in accordance with the distribution of visual object-selective cells reported in previous studies in which object recognition was emphasized (O'Scalaidhe et al. 1997; Wilson et al. 1993). However, because visual objects were all presented at the fovea in our study, the ventral distribution of object-selective cells may reflect an emphasis of the central visual field in the lateral PFC. On the other hand, we found that rank-selective cells were located dorsal to the principal sulcus. Because the rank selectivity had to be derived from the processing of object information, the specification of rank order required more advanced processing of visual information than required for the detection of object features. This finding, therefore is in line with the two-stage hypothesis proposed by Petrides: first stage in the ventral and second stage in the dorsal PFC (Petrides 1991, 1994). Furthermore, it appears that rank order information is further combined with object-selective information in cells in the ventral portion of the lateral PFC, where we found cells to be selective for the combination of the rank and physical properties of objects: third-stage processing. Thus the present study endorses the importance of integrative processing of information in the LPFC, a notion proposed by Rao et al. (1997), who reported the convergence of object and spatial information in individual cells.

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GRANTS

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