SHORT COMMUNICATION
Delay-period activities in two subdivisions of monkey inferotemporal cortex during pair association memory task

Yuji Naya,[1,2] Masatoshi Yoshida,[1,2] Masaki Takeda,[1] Ryoko Fujimichi[1] and Yasushi Miyashita[1,2]

[1]Department of Physiology, The University of Tokyo School of Medicine, Hongo, Tokyo 113–0033, Japan
[2]Laboratory of Cognitive Neuroscience, National Institute for Physiological Sciences, Okazaki, Aichi 444–8585, Japan

Abstract

The macaque inferotemporal cortex, which is involved in encoding and retrieval of visual long-term memory, consists of two distinct but mutually interconnected areas: area TE (TE) and area 36 (A36). In the present study, we compared delay-period activities of the two subdivisions in terms of their signal contents. We recorded single-unit activities from TE and A36 during a delayed pair association task, in which monkeys were required to choose the paired associate of a cue stimulus after a delay period. The stimulus-selective delay-period activities of single neurons were characterized by using partial correlation coefficients of delay-period activities for each cue stimulus with the cue-period responses to that stimulus (cue-holding index, CHI) and with the cue-period responses to its paired associate (pair-recall index, PRI). The delay-period activities of TE neurons preferentially represented the paired associate (PRI, median = 0.54) rather than the cue stimulus itself (CHI, 0.23) ($P < 0.001$, $n = 70$), while the delay-period activities of A36 neurons retained both the cue stimulus and its paired associate equivalently (CHI, 0.44; PRI, 0.46) ($P = 0.78$, $n = 38$). These results indicate that the signal contents of delay-period activities differ between the two subdivisions: TE mostly represents a sought target that is retrieved from long-term memory, while A36 in addition retains cue-stimulus that is transmitted from earlier visual areas.

Introduction

The inferior temporal (IT) cortex consists of two anatomically distinct areas: area TE (TE) and area 36 (A36) (Squire & Zola-Morgan, 1991; Suzuki & Amaral, 1994; Saleem & Tanaka, 1996; Mishkin *et al.*, 1997). TE is a unimodal neocortical area located at the final stage of the ventral visual pathway, which processes object vision (Tanaka, 1996; Sheinberg & Logothetis, 1997; Janssen *et al.*, 2000; Baylis & Driver, 2001). On the other hand, A36 is a limbic polymodal association area and a component of the medial temporal lobe memory system (Mishkin *et al.*, 1997; Rolls, 2000).

There have been a number of previous studies of neuronal responses in the subdivisions of the IT cortex. Most of these studies compared visual responses to objects in TE with those in A36 and demonstrated differential responses with respect to recency of stimulus, reward association and stimulus–stimulus association (Xian & Brown, 1998; Liu & Richmond, 2000; Messinger *et al.*, 2001; Naya *et al.*, 2003). In contrast, there have been few studies comparing delay-period activities in the two areas, although IT neurons are well known to show stimulus-selective activity in periods when no physical visual stimulus is present. These previous studies on delay-period activities did not distinguish between TE and A36, but they reported that the delay-period activities in the IT cortex reflected either a cue stimulus itself, that is, perception-derived information from the early visual areas (Fuster & Jervey, 1982; Miyashita & Chang, 1988; Yakovlev *et al.*,

1998; Erickson & Desimone, 1999) or a sought target, that is, information retrieved from long-term memory (LTM) (Sakai & Miyashita, 1991; Naya *et al.*, 1996; Erickson & Desimone, 1999). The contribution of the sought target was most clearly demonstrated by a modified version of pair association (PA) task (Naya *et al.*, 1996). In our previous study (Naya *et al.*, 2001), we compared the delay-period activity in TE with that in A36, and found backward spread of LTM-derived information between the two areas. However, we did not examine the delay-period activity related directly to the cue stimulus (i.e. perception-derived information) in that study.

In the present study, we examined whether delay-period activities of the two subdivisions of the IT cortex represent a cue stimulus or its paired associate in a PA task, and found a dissociation of the signal contents of the delay-period activities between TE and A36.

Materials and methods

Subjects and behavioural task

The experiments were performed in three adult monkeys (*Macaca fuscata*; 6.0–9.0 kg). Head bolts and a chamber for microelectrode recording were attached to the skull under aseptic conditions and general anaesthesia with sodium pentobarbital (25 mg/kg body weight per hour, i.v.). The care and use of the animals conformed to the regulations of the National Institute for Physiological Sciences, Japan and the NIH Guide for the Care and Use of Laboratory Animals. The three animals in the present study were also used in our previous study (Naya *et al.*, 2003).

The detailed procedure for the PA task was described previously (Naya *et al.*, 2003). Visual stimuli were 24 monochrome Fourier

descriptors extending approximately $5° \times 5°$. In each trial, one cue stimulus and then two choice stimuli were presented sequentially with a delay of 2.0 s. The choice stimuli were the paired associate of the cue stimulus (target) and one from a different pair (distracter). The monkey was rewarded with fruit juice for touching the correct target.

Electrophysiology and data analysis

Extracellular discharges of single neurons were recorded with a glass-insulated tungsten microelectrode from one hemisphere in each of the three monkeys (Naya *et al.*, 2003). Each location of the electrode track was measured using X-ray imaging. After the experiments, the recording sites were reconstructed histologically based on three to four electrolytic-marking lesions and three dyes injected as markers. The border between TE and A36 was determined based on the cytoarchitecture (Suzuki & Amaral, 1994; Saleem & Tanaka, 1996; Tokuyama *et al.*, 2000; Yoshida *et al.*, 2003).

We defined cue-period response as the firing rate from 60 to 320 ms after cue onset. The firing rate from 1320 to 2320 ms after cue onset (1.0–2.0 s after cue offset) was measured as delay-period activity to exclude visual off response and examine the activity that reached a stationary state. The stimulus selectivity of neuronal activities for the 24 cue stimuli was tested during either the cue or delay period by one-way analysis of variance (ANOVA).

The delay-period activities for the set of 24 cue stimuli were denoted as a 24-dimensional vector **D**: $[d_1, \cdots, d_{24}]$ or **Dp**: $[d_{p(1)}, \cdots, d_{p(24)}]$. The cue-period responses were denoted as **C**: $[c_1, \cdots, c_{24}]$ or **Cp**: $[c_{p(1)}, \cdots, c_{p(24)}]$, where the *i*-th and p(*i*)-th stimuli belong to a pair. To define the cue-holding index (CHI) and the pair-recall index (PRI), partial correlation coefficients of **D** with the cue-period responses to the

TABLE 1. Percentages of the cue-selective neurons which were delay-selective in areas TE and 36

Monkey 1		Delay-selective neurons (% of cue selective)		
		Monkey 2	Monkey 3	Total
Area TE	35 (116)	19 (84)	11 (132)	21 (332)
Area 36	70 (37)	33 (21)	36 (14)	53 (72)

The total numbers of cue-selective neurons in each area are shown in parentheses.

corresponding cue stimuli **C** and to the paired associate stimuli **Cp** were calculated, using standard formulae (Movshon & Newsome, 1996; Erickson & Desimone, 1999; Naya *et al.*, 2001):

$$\text{CHI} = [<C|D> - <C|C_p><C_p|D>] / \{[1 - <C|C_p>^2][1 - <C_p|D>^2]\}^{1/2},$$

$$\text{PRI} = [<C_p|D> - <C|C_p><C|D>] / \{[1 - <C|C_p>^2][1 - <C_p|D>^2]\}^{1/2},$$

where $<A|B>$ indicates a simple correlation coefficient between A and B. Note that $<C|D> = <C_p|D_p>$ and $<C_p|D> = <C|D_p>$. CHI and PRI values are bounded by -1 and 1. If a single neuron in a population showed the pattern of stimulus selectivity during the delay
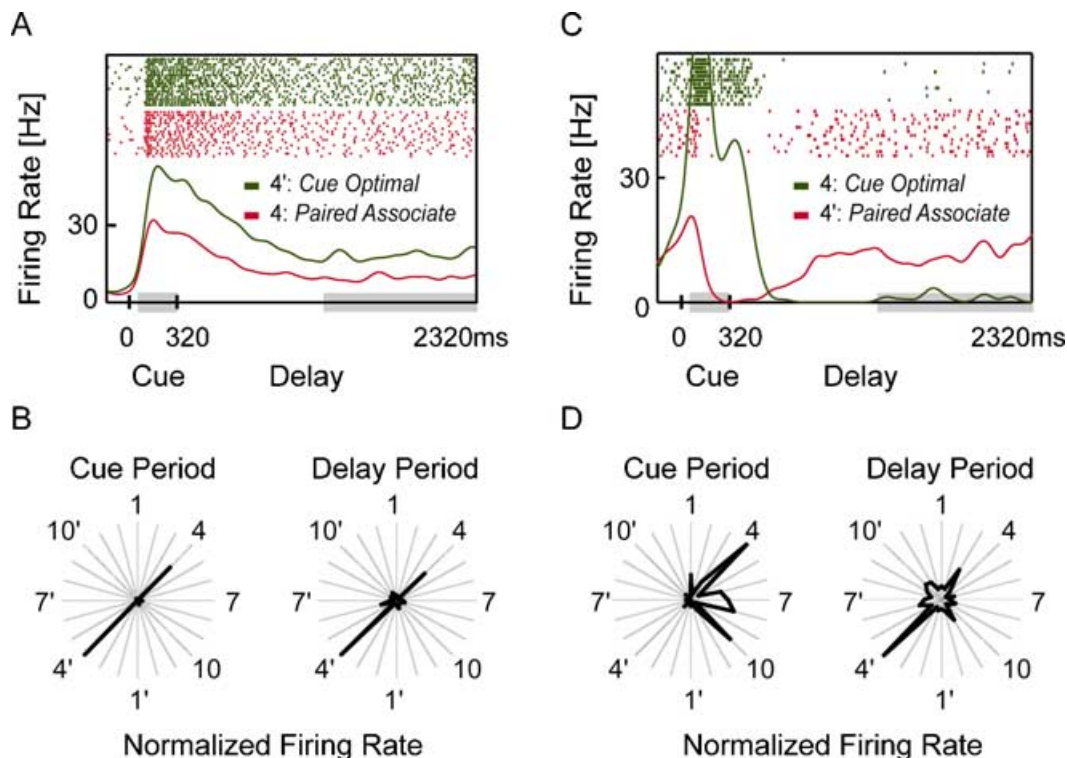

FIG. 1. Examples of delay-selective neurons from A36 (A and B) and TE (C and D). (A and C) For the raster displays and spike density functions, trials using the cue-optimal stimulus as a cue (dark green) and those using the paired associate as a cue (red) were aligned at the cue onset. The responses during the periods within the grey boxes (cue period, 60–320 ms; delay period, 1320–2320 ms) were examined in the following analyses (B, D and Fig. 2). (B and D) Mean discharge rates during the cue (left) and the delay periods (right) are shown in polar plots for each cue presentation. The responses to stimuli 1, 2. . . and to their paired associates, stimuli 1′, 2′. . ., are indicated by radial lines. The discharge rates were normalized based on the maximum values for each period (B, cue period, 44.0 Hz at stimulus 4′, delay period, 18.6 Hz at stimulus 4′; D, cue period, 48.7 Hz at stimulus 4, delay period, 18.7 Hz at stimulus 4′).

period that was independent of the pattern of stimulus selectivity during the cue period, the mean values of the PRI and the CHI for the neuronal population would be expected to approach zero as the number of neurons in the population increased. These measures are useful, because an IT neuron has a tendency to encode both of the paired stimuli (Sakai & Miyashita, 1991; Erickson & Desimone, 1999; Messinger *et al.*, 2001; Naya *et al.*, 2003) and because this correlation between **C** and **Cp** must be removed in the multiple regression analysis of **D**.

Results

In total, 404 neurons, which showed a significant stimulus-selective response ($P < 0.01$, one-way ANOVA) during the cue period (60–320 ms from cue onset), were recorded from TE (332 neurons) and A36 (72 neurons) of the three monkeys performing the PA task, which constituted the database of this study (Table 1). These neurons are hereafter referred to as cue-selective neurons. Among them, a total of 108 neurons showed significant stimulus-selective activity ($P < 0.01$) during the delay period (1.0–2.0 s from cue offset), and are hereafter referred to as delay-selective neurons (70 neurons in TE and 38 neurons in A36). The percentage of the delay-selective neurons was significantly larger in A36 (53% of the cue-selective neurons) than TE (21%) ($P < 0.001$, χ^2-test).

Cue-holding activity and pair-recall activity

The responses of a representative delay-selective neuron, with sustained activity related to a cue stimulus itself, are shown in Fig. 1A. One stimulus elicited the strongest response during the cue period and maintained the highest tonic activity throughout the delay period (Fig. 1A, dark green). The second-best cue-optimal stimulus, which was the paired associate of the cue-optimal stimulus used here, also elicited sustained activity (Fig. 1A, red). The paired associate of the cue-optimal stimulus is hereafter referred to as the pair stimulus. In this neuron, the pattern of stimulus selectivity during the delay period was similar to that during the cue period (Fig. 1B). We refer to this type of activity as cue-holding. In addition to the neurons with cue-holding activity, we found another type of delay-selective neuron with delay-period activity related to the paired associate specified by a cue stimulus, rather than to the cue stimulus itself (Fig. 1C). In this type, the peak response elicited by the cue-optimal stimulus was attenuated after cue presentation (Fig. 1C, dark green). When the pair stimulus was used as a cue, the neuron started to respond in the middle of delay period and maintained its tonic activity until choice stimuli were presented (Fig. 1C, red). The pair stimulus elicited the highest delay-period activity among the stimuli tested (Fig. 1D). We refer to this type of activity as pair-recall (Naya *et al.*, 1996).

Population analysis

We examined the cue-holding and pair-recall activities of TE and A36 neurons at the population level. For the delay-selective neurons, the delay-period activities to the cue-optimal stimulus were compared with those to the pair stimulus (Fig. 2A). In TE, the delay-period activities to the pair stimulus (11.0 ± 0.8 Hz) were significantly larger than those to the cue-optimal stimulus (8.3 ± 0.7 Hz) ($P < 0.001$, paired *t*-test). On the other hand, in A36, the delay-period activities to the pair stimulus (7.7 ± 0.8 Hz) did not differ from those to the cue-optimal stimulus (7.1 ± 0.7 Hz) ($P = 0.41$).

We further characterized the cue-holding and pair-recall activities by considering responses to all cue stimuli. For each neuron, we calculated the partial correlation coefficients of delay-period activity with the cue responses to that stimulus (cue-holding index; CHI) and to
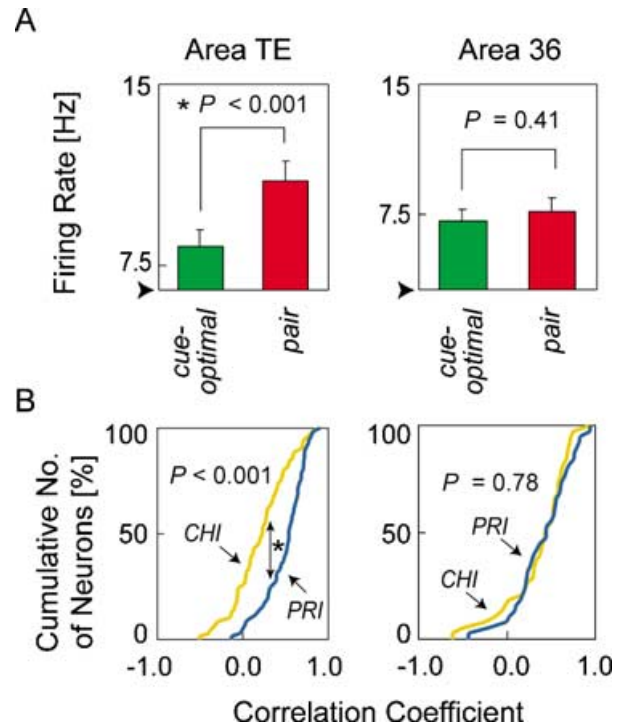

FIG. 2. Characterization of delay-period activity in TE and A36. (A) The averages of the delay-period activities to the cue-optimal (green) and the pair stimulus (red) for the delay-selective neurons in TE (left, $n = 70$) and A36 (right, $n = 38$). Error bars denote SE. Arrowheads denote the averaged responses to other pairs of stimuli. The delay-period activities to the pair stimulus were significantly larger than those to the cue-optimal stimulus in TE (asterisk, $P < 0.001$, paired *t*-test), but not in A36 ($P = 0.41$). The delay-period activities to either the cue-optimal stimulus or the pair stimulus were significantly larger than the averaged delay-period activities to the other pairs of stimuli in both areas. (B) Cumulative frequency histograms of the CHI (yellow) and the PRI (blue) for the delay-selective neurons in TE (left) and A36 (right). The PRIs were significantly higher than the CHIs for the delay-selective neurons in TE (asterisk, $P < 0.001$, Kolmogorov–Smirnov test), but not in A36 ($P = 0.78$). The delay-selective neurons showed significantly positive CHIs and PRIs in both areas.

its paired associate (pair-recall index; PRI) (see Materials and methods). A large CHI implied cue-holding activity as shown in Fig. 1A and B (CHI = 0.95, PRI = −0.47), while a large PRI implied pair-recall activity as shown in Fig. 1C and D (CHI = −0.01, PRI = 0.64). The values of CHI and PRI were compared in neuronal populations of the two areas. In TE, the PRIs of the delay-selective neurons (median, 0.54) were significantly higher than their CHIs (median, 0.23); $P < 0.001$, Kolmogorov–Smirnov test (Fig. 2B, left). On the other hand, in A36, the PRIs of the delay-selective neurons (median, 0.46) were not different from their CHIs (median, 0.44); $P = 0.78$ (Fig. 2B, right). Moreover, the CHIs of the delay-selective neurons in TE were significantly lower than those in A36 ($P < 0.01$), while the PRIs of the delay-selective neurons were not significantly different between the two areas ($P = 0.25$).

The finding that A36 showed high CHI values as well as high PRI values could be due to single neurons that had high values for both CHI and PRI. We examined this possibility by calculating the correlation coefficient between CHI and PRI values of single neurons across the population of delay-selective A36 neurons. We found that CHI and PRI values of the A36 neurons negatively correlated ($r = -0.53$, $P < 0.001$), which indicated that high CHI and PRI values in A36 were not supported by single neurons that had high values for

both indices, but were supported by a set of neurons, some with high CHI and low PRI values and some others with low CHI and high PRI values.

Discussion

The results of the present study indicate that the signal contents of delay-period activity differ between two subdivisions of the IT cortex, TE and A36. TE neurons mostly represented the paired associate specified by the cue stimulus (median in CHI, 0.23; median in PRI, 0.54). On the other hand, in A36, both the cue-stimulus and its paired associate were retained equivalently (median = 0.44 in CHI; median = 0.46 in PRI). This equivalent retention in A36 was supported by a set of neurons, some with high CHI and low PRI values and some other with low CHI and high PRI values.

In previous studies, the delay-period activities of IT neurons were examined using a delayed matching-to-sample task, in which monkeys were required to choose the same picture as a sample stimulus (Fuster & Jervey, 1982; Miyashita & Chang, 1988; Yakovlev *et al.*, 1998). In the delayed matching-to-sample task, the delay-period activity coupled with the sample stimulus cannot be distinguished from that coupled with the expected choice stimulus. The PA task has the advantage that the cue stimulus is different from the expected choice stimulus. This allowed us to detect differences in the signal contents of delay-period activity between TE and A36.

In the present study we successfully found differential patterns of signal contents, but they were somewhat unexpected in nature. Since perceptual information from early visual areas is transmitted to TE before A36, the neural network of TE, rather than A36, would be expected to represent a cue-stimulus. On the other hand, since A36 is a limbic association area that connects with the hippocampus through the entorhinal cortex, the neural network of A36, rather than TE, would be expected to represent the paired associate of the cue stimulus. Nevertheless, these predictions did not hold true. The unexpected results in the present study may be partially explained by the backward transmission of the retrieved information from A36 to TE (Naya *et al.*, 2001). However, the present study indicates that, in contrast to the LTM-derived information, the perception-derived information in the neural network of A36 is not transmitted backward to the neural network of TE. A reasonable interpretation of this finding may be that the backward signal transmission in the IT cortex is equipped with a selective gating mechanism, which preferentially passes information of a sought target (Naya *et al.*, 1996). Another interpretation may be that there are some mechanisms of actively inhibiting irrelevant information in TE. This interpretation was not testable in the present study because the active inhibition was not distinguished from the passive decay of the visual off response. In either interpretation, we suggest that activities of TE are influenced by the behavioural demand, while activities of A36 are for the most part automatically driven.

Acknowledgements

Abbreviations

A36, area 36; CHI, cue-holding index; IT, inferior temporal; LTM, long-term memory; PA, pair association; PRI, pair-recall index; TE, area TE.

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