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Kuniyoshi Sakai & Yasushi Miyashita

Department of Physiology, School of Medicine, University of Tokyo,
7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

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Department of Physiology, School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

MOST of our long-term memories of episodes or objects are organized so that we can retrieve them by association. Clinical neuropsychologists assess human memory by the paired-associate learning test, in which a series of paired words or figures is presented and the subject is then asked to retrieve the other pair member associated with each cue[1]. Patients with lesions of the temporal lobe show marked impairment in this test[2–6]. In our study, we trained monkeys in a pair-association task[7] using a set of computer-generated paired patterns. We found two types of task-related neurons in the anterior temporal cortex. One type selectively responded to both pictures of the paired associates. The other type, which had the strongest response to one picture during the cue presentation, exhibited increasing activity during the delay period when the associate of that picture was used as a cue. These results provide new evidence that single neurons acquire selectivity for visual patterns through associative learning. They also indicate neural mechanisms for storage and retrieval in the long-term memory of paired associates.

We prepared 24 computer-generated pictures for each monkey, and sorted geometrically distinct patterns into pairs (Fig. 1*a*). The combination of the paired associates is not predictable without memorizing them beforehand. Two macaque monkeys (*Macaca fuscata*) were trained to memorize a set of 12 pairs through repeated trials in the pair-association task. In each trial, a cue stimulus was presented on a video monitor for 1 s (Fig. 1*b*). After the delay period (4 s), a choice of two stimuli, the paired associate of the cue and one from a different pair, was shown. The monkey obtained fruit juice as a reward for correctly touching the paired associate within 1.2 s. In the recording sessions after training (Fig. 1 legend), the monkeys' performance was 70–100% correct. Extracellular spike discharges of single neurons were recorded from the anterior part of the temporal cortex (Fig. 2*a*), as reported in previous studies[8,9].

Figure 2 shows one type of neuron with picture-selective responses during the cue period. One picture elicited the strongest response during the cue period from a neuron, with some activity during the delay period (Fig. 2*b*). By contrast, another picture elicited no response at all (Fig. 2*c*). This neuron responded reproducibly to only a few pictures, but not to other pictures in the set. It might be that the cell responded to geometrically similar patterns. The strongest and the second-strongest responses were ascribed to a particular pair which had no apparent geometrical similarity (Fig. 2*d*). Some other cells showed broader tuning and responded to more than three pictures. Nevertheless, paired pictures were found to be among the most effective stimuli for these cells (Fig. 2*e*, *f*). We call this type of cell a 'pair-coding neuron', which manifests selective cue responses to both pictures of the paired associates.

Of 577 isolated neurons, 91 cells reproducibly showed a strong picture-selective response during the cue period (Fig. 2 legend). The most effective stimuli for the 91 cells covered all pictures in the set. These responsive cells tended to be located near to one another (1–2 mm wide) in the temporal cortex. Thirty-two of the 91 cells responded to only one picture, whereas 59 cells responded to more than two pictures. We further analysed these 59 cells by calculating two coupling indices for each neuron (see legend to Fig. 2). One coupling index (denoted as CI_p) measures correlated neural responses to paired associates, whereas the other coupling index (CI_r) estimates responses to other random combinations among 24 pictures. The latter index CI_r serves as an experimental control for untrained association between two pictures. For each cell, we defined a pair index (PI) as equal to $CI_p - CI_r$. The frequency distribution of PI values is shown in Fig. 2*g*, demonstrating that the paired associates elicited significantly correlated responses ($P < 0.015$; Wilcoxon's signed-rank test, $n = 59$). We conclude that the selectivity of these neurons was acquired through learning

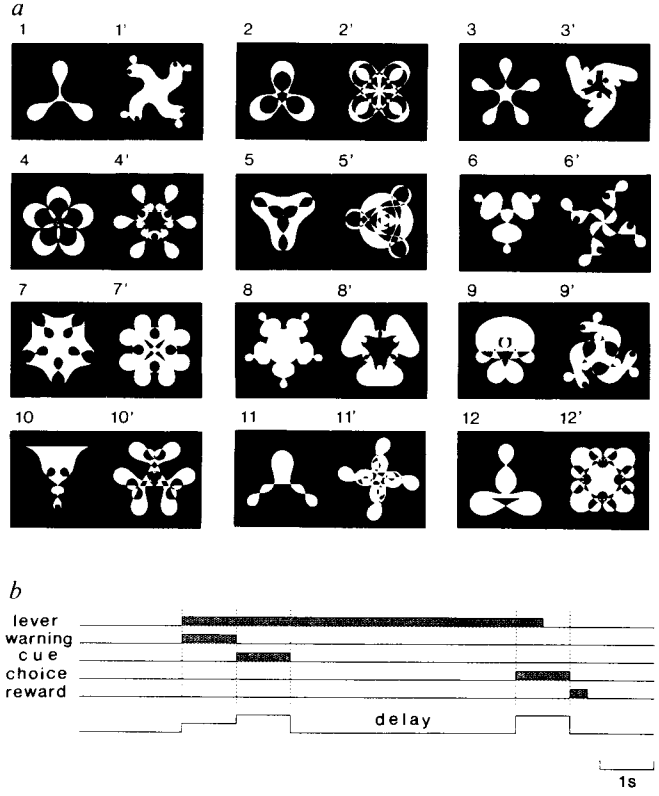

FIG. 1 Pair-association task for monkeys used to assess long-term memory. *a*, 12 pairs of Fourier descriptors (1 and 1'–12 and 12') for stimuli in the task. The monkeys learned to retrieve the other member of the pair associated with each cue picture. *b*, Sequence of events in a trial. Lever, lever press by the monkey to initiate a new trial; warning, green square (1 s); cue, one of 24 pictures as a cue stimulus (1 s); delay, green square (4 s); choice, a choice of two stimuli, the paired associate of the cue and one from a different pair; reward, fruit-juice reward for correctly touching the associate. Bottom trace, events chart used in Figs 2*b*, *c* and 3.

METHODS. Fourier descriptors were generated according to the reconstruction theorem with specified sets of harmonic amplitudes and phase angles[23]. The real images on a video monitor were yellow monochrome against a black field. This set was used for one monkey, and coloured fractal patterns[8] were used for the other. Sorting the pictures into pairs is basically random, avoiding apparent geometrical resemblances such as rotational symmetry. In each trial, cue stimulus was presented in the centre of the monitor screen and stimuli for choice were shown randomly in two of four positions (arranged in two rows of two columns). If the monkey released the lever before the choice, that trial was aborted. Error trials were not included in the rastergrams of Figs 2*b*, *c* and 3. The collected trials shown in these figures were originally separated by intervening trials of other cue stimuli, and were sorted by off-line computation. Training procedures of this pair-association task are as follows. Twelve pairs used in neuronal recordings were divided into three blocks (four pairs per block). After separate learning of three blocks, all 12 pairs were presented in random order. The direction of association (for example from 1 to 1', or from 1' to 1) was randomized except for the early training phase. Delay interval was 0.5 s before thorough randomization, and was increased to 4 s. Error correction trials were included in the early training phase. The criterion for acquisition was two consecutive days of 26 correct responses in 30 trials (87%). The two monkeys took 876 ± 303 trials per picture (mean ± s.e.m.) to reach this criterion. Extracellular spike discharges of single neurons were recorded using standard physiological techniques[24].

of the pair-association task.

We found another type of neuron with picture-selective activities during the delay period. One picture elicited the strongest response during the cue period from a single neuron (Fig. 3*a*). In the trial when the paired associate of this cue-optimal picture was used as a cue, the same cell had the highest tonic activity during the delay period in contrast to a weak response during the cue period (Fig. 3*b*). This delay activity gradually increased until the choice of stimuli appeared. Furthermore, the paired associate of the second-best cue-optimal picture still elicited a sustained activity during the delay period (Fig. 3*c*, *d*). Other pictures evoked weak or no response (Fig. 3*e*, *f*). The delay activities were confined to a few cue stimuli in the set. We call this type of cell a 'pair-recall neuron', in which the paired associate of a cue-optimal picture elicited the highest delay activity.

Eleven of 91 cells showed picture-selective delay activities that surpassed cue responses in those trials. Out of 11 cells, 10 were pair-recall neurons as defined above. The highest delay activity of the pair-recall neurons does not represent mere sensory after-discharge, because it is stronger than the cue response. Furthermore, a significant augmentation of discharge rates was observed for the highest delay activity when mean discharge rates at two intervals were compared: 200–1,400 ms (near the beginning of the delay interval) and 2,760–3,960 ms (near the end) after delay onset ($P<0.05$; Wilcoxon's signed-rank test, $n=11$). By contrast, the delay activity elicited by a cue-optimal picture itself was significantly reduced during the delay period ($P<0.005$; $n=11$).

Out of 91 responsive neurons, 18 cells showed more than three error trials (where the monkey made the incorrect choice) in which a cue-optimal picture was presented. There was no significant difference in mean discharge rates during the cue period between correct and error trials ($P>0.05$; Wilcoxon's signed-rank test, $n=18$). Out of 10 pair-recall neurons, two cells exhibited more than three error trials in which the paired associate of a cue-optimal picture was presented. One cell showed no significant difference, whereas the other cell showed a higher

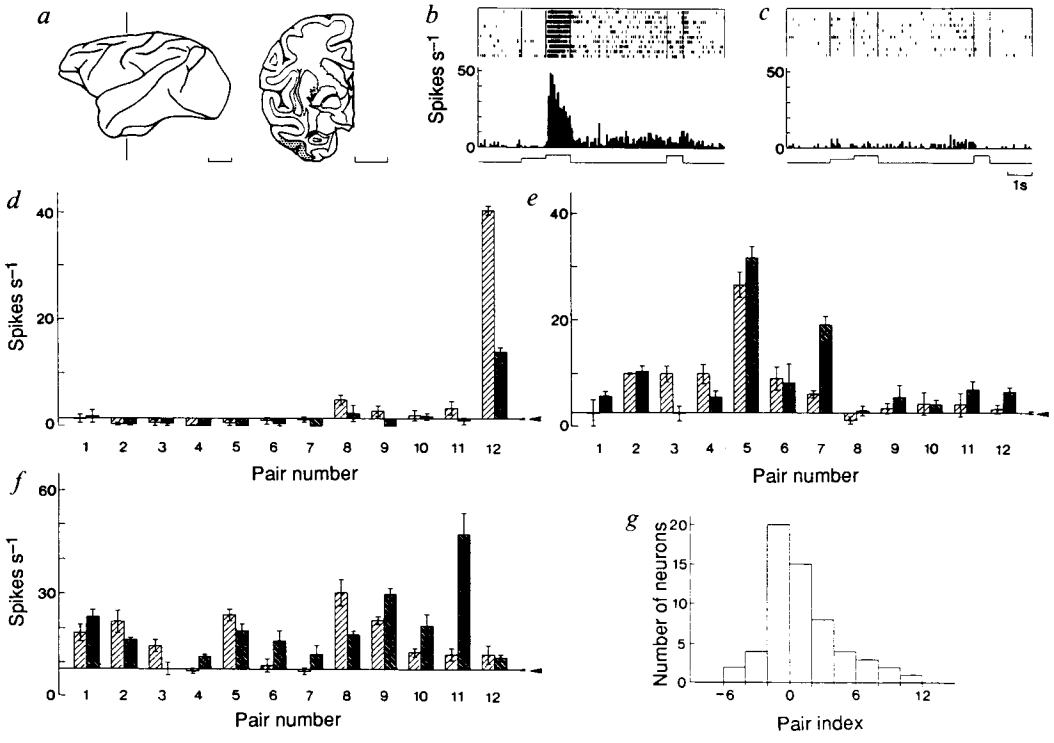

FIG. 2 Responses of 'pair-coding neurons' which were selective to both pictures of the paired associates. *a*, Location of recorded neurons. Left, lateral view of a monkey brain. Right, cross-section indicated by a vertical line on the lateral view. The stippled area represents the range of recording sites. Scale bars, 10 mm. *b*, Rastergrams of neural discharges in each trial (upper) and spike-density histograms (lower) obtained from a single neuron. Bin width, 80 ms. These trials were collected for cue 12 which elicited the strongest response during the cue period. *c*, Trials for cue 7 which elicited no response at all in the same cell as *b*. *d*, Mean discharge rates for each cue presentation (mean ± s.e.m.) relative to the spontaneous discharge rate (denoted by an arrowhead) in the same cell as *b* and *c*. Cue stimuli are labelled as 'Pair number' on the abscissa (light histogram bar in number 1: cue 1, dark histogram bar in number 1: cue 1′ and so on). This neuron selectively responded to both pictures of the paired associates 12 & 12′. *e*, Mean discharge rates for another cell. This neuron responded optimally to both pictures of the paired associates 5 & 5′. *f*, Mean discharge rates for another cell, whose pair index (see below) was equal to the mean value (1.3). In the cells shown in *d* and *e*, pair indices were 8.7 and 6.5, respectively. *g*, Frequency histogram showing the distribution of the pair indices in 59 responsive neurons in two monkeys. Positive values indicate that the neurons exhibited more correlated responses to paired associates than other random combinations, whereas negative values indicate the converse.

METHODS. We defined two coupling indices as:

$$CI_p = N_p^{-1} \sum_{i} \sum_{\substack{j \\ i<j}} \frac{(x_i - b)(x_j - b)}{(x_{\text{best}} - b)(x_{\text{2nd-best}} - b)} \times 100,$$

with $j=i'$ for paired associates,

$$CI_r = N_r^{-1} \sum_{i} \sum_{\substack{j \\ i<j}} \frac{(x_i - b)(x_j - b)}{(x_{\text{best}} - b)(x_{\text{2nd-best}} - b)} \times 100,$$

with $j \neq i'$ for random combinations, where x_i denotes a mean discharge rate during the cue period for the ith picture (the ith and i'th pictures belong to a pair), b is a spontaneous discharge rate, x_{best} and $x_{\text{2nd-best}}$ are mean discharge rates for the best and second-best cue-optimal pictures in each cell, N_p and N_r are the total number of combinations for two cases. Pair index (PI) was then defined as:

$$PI = CI_p - CI_r.$$

Evaluation of cue responses was done by calculating a mean discharge rate for each picture as follows. Spike numbers were collected over 400 ms at the beginning of the cue interval. They were averaged across trials for the same cue stimulus and their variances were evaluated to test reproducibility in each cell. Out of 577 isolated neurons, 436 cells were unresponsive. The pair index of weakly responsive cells is very susceptible to random fluctuations about a spontaneous discharge level. Moreover, weak responses could not be ascribed to the optimal stimulus[25]. We therefore examined 104 cells whose discharge rates ($x_{\text{best}} - b$) were distributed beyond 15.5 Hz (the leftmost saddle point in the distribution of 141 responsive cells). Out of the 104 cells, 13 showed nonselective responses to all pictures, whereas the other 91 cells were picture-selective.

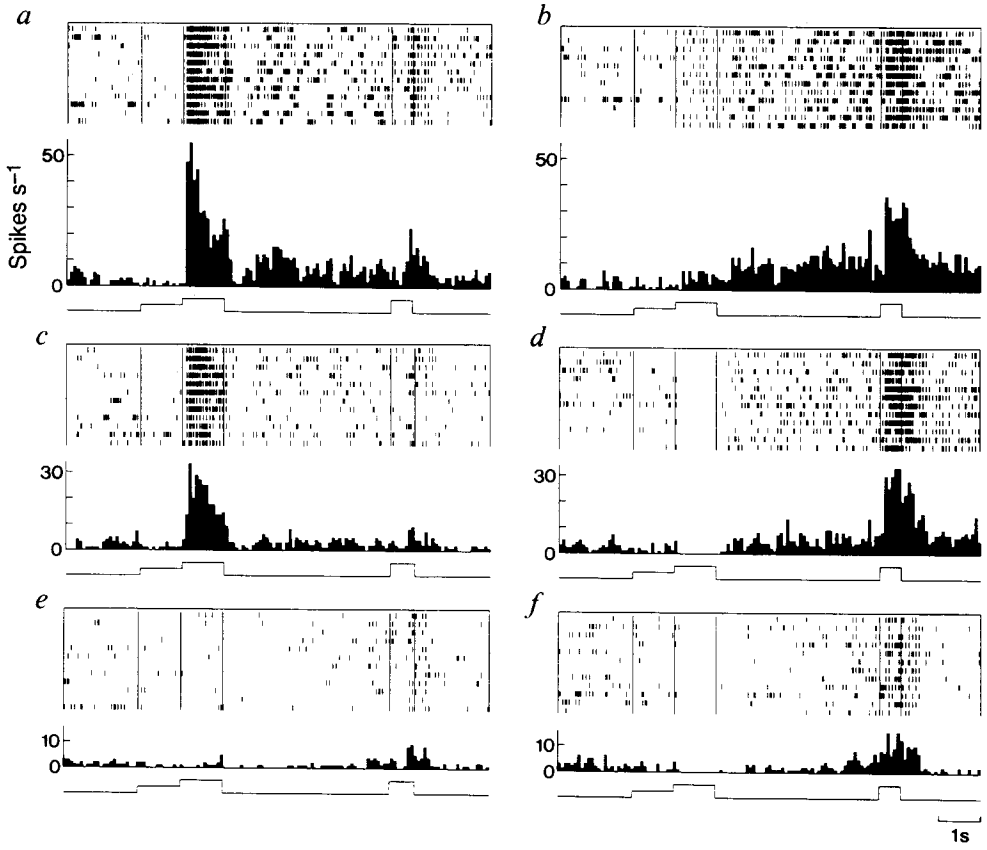

FIG. 3 Responses of a 'pair-recall neuron' which exhibited picture-selective activity during the delay period, presumably reflecting retrieval of the paired associate. *a–f*, Rastergrams and spike-density histograms obtained from a neuron. Bin width, 80 ms. *a*, Trials for cue 12 which elicited the strongest cue response. *b*, Trials for cue 12'. Note the tonic increasing activity during the delay period, which is much higher than the cue response. *c*, Trials for cue 1 that resulted in the second-strongest cue response. *d*, Trials for cue 1'. Note the sustained delay activity and the inhibitory cue response. *e*, Trials for cue 3 that elicited no response. *f*, Trials for cue 3'. In *f*, the discharges during the choice period are due to stimuli for choice other than 3, such as 1.

delay activity in correct trials than in error trials, which correlated with the monkey's choice ($P < 0.01$; two-tailed modified t test[10], $t' = 3.21$ with 17 d.f.).

We previously reported that a picture-selective delay activity reflected stimulus–stimulus association caused by the fixed order of picture presentation in a visual delayed matching-to-sample task[9]. But evidence from that experiment is restricted to implicit learning because the monkey could solve the task without memorizing the sequence. In the present task, associative learning was imposed to assess the long-term memory more directly.

In the primate inferior temporal cortex and part of the superior temporal sulcus, neural responses to complex objects such as faces[11,12], hands[12] and Fourier descriptors[13] have been reported. According to our results, acquired pairing is now regarded as an important coding faculty. The properties of pair-coding neurons indicate that memory storage is organized such that single neurons can code both pictures of the paired associates. A possible basis for this coding lies in the change of synaptic connections through repetitive learning[14,15], whereby two pictures are always paired with each other.

Anticipatory neural activities that precede the initiation of movements and increase during the preparatory period have been reported in the primate frontal cortex[16–18]. In our pair-association task, the increasing delay activity of pair-recall neurons is not related to motor response because the monkey could not predict which position should be touched. As noted before, this delay activity is not only picture-selective, but also closely coupled with the paired associate that is not actually seen but retrieved. The neural mechanism for the retrieval process remains to be identified, but it may well involve the pair-recall neurons.

A recent lesion study has demonstrated that monkeys with bilateral removal of hippocampus and amygdala do not relearn the pair-association task in the training limit[7]. The type of memory this task used would therefore correspond to one that relies on the integrity of these structures. The medial temporal region is considered essential to the memory consolidation process, by which certain evanescent information obtains an enduring representation in long-term memory[19,20]. As the anterior temporal cortex we studied links the visual system and the limbic memory system[21,22], the unique neurons described here could serve as memory storage elements, also activated in the retrieval process. □

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