Consolidation of Visual Associative Long-Term Memory in the Temporal Cortex of Primates

Yasushi Miyashita,*† Masashi Kameyama,* Isao Hasegawa,* and Tetsuya Fukushima*

*Department of Physiology, The University of Tokyo School of Medicine, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan; and †Mind Articulation Project, ICORP, Japan Science and Technology Corporation, Yushima, Tokyo 113, Japan

Neuropsychological theories have proposed a critical role for the interaction between the medial temporal lobe and the neocortex in the formation of long-term memory for facts and events, which has often been tested by learning of a series of paired words or figures in humans. We have examined neural mechanisms underlying the memory “consolidation” process by single-unit recording and molecular biological methods in an animal model of a visual pair-association task in monkeys. In our previous studies, we found that long-term associative representations of visual objects are acquired through learning in the neural network of the anterior inferior temporal (IT) cortex. In this article, we propose the hypothesis that limbic neurons undergo rapid modification of synaptic connectivity and provide backward signals that guide the reorganization of neocortical neural circuits. Two experiments tested this hypothesis: (1) we examined the role of the backward connections from the medial temporal lobe to the IT cortex by injecting ibotenic acid into the entorhinal and perirhinal cortices, which provided massive backward projections ipsilaterally to the IT cortex. We found that the limbic lesion disrupted the associative code of the IT neurons between the paired associates, without impairing the visual response to each stimulus. (2) We then tested the first half of this hypothesis by detecting the expression of immediate-early genes in the monkey temporal cortex. We found specific expression of zif268 during the learning of a new set of paired associates in the pair-association task, most intensively in area 36 of the perirhinal cortex. All these results with the visual pair-association task support our hypothesis and demonstrate that the consolidation process, which was first proposed on the basis of clinico-psychological evidence, can now be examined in primates using neurophysiological and molecular biological approaches.

Key Words: memory consolidation; inferior temporal cortex; perirhinal cortex; pair association; ibotenic acid; immediate-early genes; transcription factor; zif268.

The cerebral cortex is organized so that hierarchically different subareas communicate by forward and backward neuronal projections (Van Essen et al., 1992). Neuropsychological studies have proposed that the backward connections from the medial temporal lobe to the neocortices play a role in the formation of long-term memory for facts and events (declarative memory) (Mishkin, 1982; Squire, 1987). Behaviorally, the declarative memory has often been tested by learning of a series of paired words or figures in humans (Squire,
We have been examining the neural mechanisms underlying declarative memory by single-unit recording with an animal model of a visual pair-association task in monkeys. In our previous studies, we provided several lines of evidence indicating that long-term representations of visual objects are acquired through learning in the neural network of the anterior inferior temporal (IT) cortex in primates (Miyashita, 1993; Miyashita, 1995; Higuchi & Miyashita, 1996; Naya, Sakai, & Miyashita, 1996). We identified two mechanisms: one is tuning and the other is association. Specifically, in the IT cortex of monkeys performing the visual pair-association task, we found a group of neurons that manifested selective responses to both of the paired associates (pair-coding neurons). It provides strong evidence that single IT neurons acquire stimulus selectivity through associative learning (Miyashita, 1993; Sakai & Miyashita, 1991).

In this article, we propose the hypothesis that limbic–neocortical interactions are critical in the consolidation process since limbic neurons undergo rapid modification of synaptic connectivity and provide backward signals that guide the reorganization of neocortical neural circuits. Two experiments were carried out to test the hypothesis. First, we tested the role of the backward signals from the medial temporal lobe to the IT cortex. Ibotenic acid was injected unilaterally into the entorhinal and perirhinal cortices, which provided massive backward projections ipsilateral to the IT cortex. We found that (i) in spite of the lesion, the sampled neurons responded strongly and selectively to the visual patterns and (ii) the paired associates failed to elicit significantly correlated responses in the cells tested with the lesion. The results show that the limbic lesion disrupted the associative code of the IT neurons between the paired associates, without impairing the visual response to each stimulus.

Second, we ask why limbic–neocortical interactions are so important. We hypothesize that limbic neurons undergo rapid modification of synaptic connectivity and provide backward signals that guide the reorganization of neocortical neural circuits. We then investigated the molecular basis of such rapid synaptic modifiability by detecting the expression of immediate-early genes in the monkey temporal cortex. We found strong expression of zif268 during the learning of a new set of paired associates in the pair-association task. This expression was quite selective, since we found no zif268 expression in a control condition, i.e., during the learning of a new stimulus set in a visual discrimination task. The results suggest that the expression of zif268 plays a role in the rapid synaptic modification in the inferior temporal gyrus.

EXPERIMENT 1

There is strong evidence that the IT cortex receives massive backward projections from the medial temporal lobe, especially from the perirhinal and entorhinal cortices (Van Hoesen, 1982; Webster et al., 1991). The hypothesis for the role of the backward connections in the consolidation process specifically predicts that the lesion of the perirhinal and entorhinal cortex would impair the formation of the associative code for pictures in the IT cells (Fig. 1). We now test this prediction.

We combined single-unit recording techniques with a surgically manipulated chronic monkey preparation so that individual IT neurons become devoid of the backward signals but can receive forward visual signals when visual stim-
ultrastructure are presented. We prepared a specific experimental design to run this project (Fig. 2). Backward neuronal connections from the limbic system to the IT cortex are interrupted by the lesion of the perirhinal/entorhinal cortices. However, bilateral lesion would impair the monkeys' behavior in the pair-association task (Murray et al., 1993). Thus the perirhinal/entorhinal cortices should be lesioned unilaterally. With the unilateral lesion, however, the IT cortex of the lesioned side could receive information from the contralateral IT cortex since receptive fields of IT neurons cover bilateral hemifields before the lesion. In order to remove the interhemispheric signal from the healthy contralateral inferior temporal cortex, we surgically cut the anterior commissure at the beginning of the experiment.
the lesion. In order to remove the interhemispheric signal from the healthy contralateral IT cortex, we surgically cut the anterior commissure at the beginning of the experiment. With this surgically manipulated chronic monkey preparation, we found selective loss of the ability of IT neurons to represent associations between picture pairs, while the ability of the neurons to respond to particular visual stimuli was left intact.

Methods of Experiment 1

Animals and surgical procedures. Two adult monkeys (Macaca fuscata) were used in accordance with the University of Tokyo Animal Care Committee. Surgery of the anterior commissure (AC) was carried out under aseptic conditions and anesthesia with sodium pentobarbital (approximately 25–30 mg/kg). The right hemisphere was retracted from the falx with a brain spoon. An aspirator was used to make a sagittal incision no more than 5 mm in length in the corpus callosum, entering the lateral ventricle at the level of the interventricular foramen. The AC was sectioned at the floor of the lateral ventricle lateral to the fornix.

The lesions of the entorhinal and perirhinal cortices were made by injecting a small volume of the neurotoxin, ibotenic acid. Ibotenic acid has been reported to kill cell bodies selectively while leaving fibers of passage in the underlying white matter unharmed (Olney, 1983). A Hamilton syringe needle was inserted through a guide tube under the control of microdrives. A grid of 1-μl injections of ibotenic acid (15 μg/μl, 0.03 μl/min) was made with approximately 2-mm spacing. One monkey (M) received injections at 22 tracks, and the other monkey (N) received injections at 19 tracks.

Behavioral task. The pair-association task was described previously (Sakai & Miyashita, 1991). Colored fractal patterns were used as the visual stimuli. We generated the patterns according to the algorithm described previously (Miyashita et al., 1991) and arranged them into 12 pairs. Sorting into pairs was basically random, avoiding apparent geometrical resemblances such as rotational symmetry.

In the task, each trial begins with the monkey continually pressing a lever in front of him. Following a fixation stimulus, a cue stimulus was presented in the center of the video monitor for 0.5 s. The cue stimulus was selected randomly from the 24 pictures in the set. After a delay period of 4 s, two stimuli for choice, the paired associate of the cue and one from a different pair, were shown randomly in two of four possible positions (arranged in two rows of two columns). The monkey obtained fruit juice as a reward for touching the correct paired associate within 1.2 s. If the monkey released the lever before the choice, that trial was aborted. Eye position was monitored with a magnetic search coil. The criterion for acquisition of prelesion learning (the set A stimuli) was 2 consecutive days of 26 correct responses in 30 trials. The two monkeys took 326 ± 57 trials per picture to reach this criterion. The criterion after the ibotenic acid lesion was set to be slightly easier, to 2 consecutive days of 23 correct responses in 30 trials. In relearning of the set A paired associates, 10 and 50 trials per picture were necessary for monkey M and monkey N, respectively. In learning of the new set (set B) after the lesion, one monkey (M) took 101 trials and the other (N) took 93 trials per picture to reach the criterion.
Electrophysiology and data collection. Recording and analysis of extracellular spike discharges of single neurons were described in a previous publication (Miyashita et al., 1989). After the monkey has learned the task, a head-holding device, scleral eye coil, and a recording chamber for microelectrode were implanted under aseptic conditions while the animal was anesthetized with sodium pentobarbital. The activity of single neurons was recorded with a glass-insulated tungsten microelectrode. The electrode was inserted through the intact dura along a stainless steel guide tube by means of a hydraulic microdrive manipulator (MO-95, Narishige). The action potentials of single cells were amplified, passed through band-pass filter circuits (30–10 kHz), and converted into digital pulses by a time-window discriminator (EN-611J, Nihonkoden).

At the beginning of unit recording, locations of responsive cells were mapped in the anterior IT cortex, and an area (about 2 × 3 mm, hatched box in Fig. 2) with a high density of responsive cells was identified; then all neuronal recordings were done from this cortical area both before and after the lesion was made. Each location of the electrode track was measured by X-ray imaging and verified histologically at the end of the experiments with reference to electrolytic lesion marks made by applying DC currents of 15–20 μA (tip negative) for 60–120 s.

Data analysis. Evaluation of cue responses was done by collecting spike numbers over 80–480 ms at the beginning of the cue interval for each picture and over 400 ms immediately preceding the appearance of the fixation stimulus. The neuron was regarded as responsive if the two distributions were significantly different (paired t test, p < .05).

The pair index, PI, was used to analyze pair-coding responses of these cells. The PI was defined in a previous study (Sahai & Miyashita, 1991). The response variability of the cells was evaluated before and after the lesion by a response variability index. The index was defined in each cell as the ratio of the standard deviation of firing rates among the trials for the optimum stimulus over the mean firing rate for the optimum stimulus. The larger values of this index indicate more trial-to-trial fluctuations of the responses.

Histology. At the conclusion of the experiment, the monkey was deeply anesthetized with an overdose of sodium pentobarbital (45 mg/kg, im) and then perfused through the aorta with 0.9% saline followed by 10% formalin in phosphate buffer (pH 7.4). The brain was removed from the skull and sectioned coronally at 40 μm on a freezing microtome. Serial sections were stained with cresyl violet as well as with the modified Gallyas method. We did not observe signs of gliosis, cell loss, or demyelination in the cortex of the recording sites except for some electrode tracks.

Results of Experiment 1

Two adult monkeys (M. fuscata) were trained in a pair-association task. Before learning of the task, the anterior commissure (AC) of the monkey was transected. The septal nuclei and fornix (Fx), as well as the underlying diencephalic structures, were spared. This surgery disconnected the anterior temporal cortex of each hemisphere from the other (Fig. 2), since most commissural fibers of this area traverse the AC rather than the corpus callosum (Pandya
et al., 1973; Demeter et al., 1990). After recovery, the monkey was trained with a set (set A) of the paired associates to the criterion performance level, and then extracellular spike discharges of single neurons were recorded as a prelesion control in the anterior IT cortex, as reported in previous studies (Miyashita, 1988; Miyashita & Chang, 1988; Sakai & Miyashita, 1991).

We deprived IT neurons of backward neural information by unilateral ibotenic acid lesions (see Methods) of the entorhinal and perirhinal cortices that provide massive backward projections ipsilateral to the IT cortex (Van Hoesen, 1982; Webster et al., 1991). The lesion covered both the medial and the lateral banks of the rhinal sulcus completely and most of the entorhinal and perirhinal cortex. The lesioned cortex suffered atrophy, but fibers in the underlying white matter were left unharmed.

After the ibotenic acid injection, the monkeys were trained with the preoperatively learned set (set A) and a new set of paired associates (set B) to the criterion of 2 consecutive days of 23 correct responses in 30 trials. Then extracellular spike discharges of single neurons were recorded from the same area as that in the prelesion control. Average performance for set A stimuli during neural recording was 81% (before lesion) and 80% (after lesion), and that for set B stimuli was 72% after lesion.

Prior to the ibotenic acid lesion, we examined the responses of 92 cells to the set A stimuli. The distribution of the PI in the 92 cells showed that the paired associates elicited significantly correlated responses in the control \( p < .01; \) Wilcoxon's signed-rank test, \( n = 92 \), which confirmed the conclusion of our previous report (Sakai & Miyashita, 1991). After the lesion, we examined the responses of 72 cells to the set A stimuli and those of 75 cells to the set B stimuli. The distribution of the PI of these cells showed that the paired associates did not elicit significantly correlated responses for either the set A or the set B stimuli.

Figure 3 compares the responsiveness of the cells in the three groups (two-way ANOVA and t test). The results of the two monkeys were not significantly different \( (p > .25) \) in a two-way ANOVA for any of the following indices, \( F(1, 233) = 1.11 \) for PI, \( F(1, 233) = 0.69 \) for SPON, \( F(1, 233) = 0.00 \) for MAX, \( F(1, 233) = 1.05 \) for VAR, and have been pooled. After the lesion of the entorhinal and perirhinal cortices, the cells responded to both the set A pictures (MAX, 35 \( \pm \) 2 spikes/s; mean \( \pm \) SEM, \( n = 72 \)) and the set B pictures (38 \( \pm \) 2; \( n = 75 \)), even more strongly than the control (31 \( \pm \) 1; \( n = 92 \)). However, the PI were reduced \( (p < .05) \) after the lesion (0.04 \( \pm \) 0.35 for set A, \(-0.19 \pm 0.27 \) for set B) compared to that of control (1.00 \( \pm \) 0.33). The spontaneous discharge rates (SPON) were not significantly different among the three groups \( (p < .2) \). We also tested with a response variability index (VAR) whether the cells exhibited any sign of pathological firings after the lesion. The larger values of this index indicate more trial-to-trial fluctuations of the responses (Methods). The index did not differ significantly between the control group (0.25 \( \pm \) 0.01) and the lesioned groups either with set A (0.28 \( \pm \) 0.02; \( p > .2 \)) or with set B stimuli (0.26 \( \pm \) 0.02; \( p > .6 \)). We conclude that lesion of the entorhinal and perirhinal cortices disrupted the associative code of the IT neurons between the paired associates, without impairing the visual response to each stimulus.

**EXPERIMENT 2**

Immediate-early genes (IEGs), a class of genes that show rapid and transient but protein synthesis-independent increases in transcription, have been pro-
FIG. 3. Effects of the lesion of the entorhinal and perirhinal cortices upon neuronal responsiveness in the pair-association task. (A) Pair index (PI). (B) Spontaneous discharge rate (SPON). (C) Maximum discharge rate with spontaneous discharge subtracted (MAX). (D) Response variability (VAR). These indices were compared between two groups: (1) neurons that were recorded prior to the lesion using the set A stimuli (stippled bar, \( n = 92 \)) and (2) those recorded after the lesion using the set B stimuli (hatched with thin line, \( n = 75 \)). Data are shown as means ± SEM (modified from H. Okuno & Y. Miyashita, 1996, Expression of the transcription factor Zif268 in the temporal cortex of monkeys during visual paired associate learning, European Journal of Neuroscience, 8, 2118-2128, Blackwell Science Ltd.).

posed to be involved in genomic responses in neurons related to learning and memory (Morgan & Curran, 1991). A number of IEGs encode transcription factors such as Fos, Jun, and Zif268, and these IEGs, especially zif268, have been hypothesized to play crucial roles in the transduction of neuronal electric signal into more permanent synaptic organization. For example, the induction of zif268 in the dentate gyrus is closely correlated with the induction of long-term potentiation (LTP) (Abraham et al., 1993; Worley et al., 1993). In songbirds, zif268 is strongly induced in specific regions of the auditory telencephalon when the birds hear the song of their own species, but induced only slightly when the birds hear songs of other species (Mello et al., 1992). However, there have been no reports that seek to determine the roles of IEGs in the consolidation process of cognitive memories in primates.
In the present study, we attempted to investigate brain areas in which IEGs were expressed during visual long-term memory formation in the primate. We trained monkeys to learn two different cognitive memory tasks, a visual pair-association task and a visual discrimination task. The visual pair-association task required the monkeys to memorize visual stimulus–stimulus associations (Sakai & Miyashita, 1991; Murray et al., 1993). The other task, the visual discrimination task, required them to memorize stimulus–reward associations (Iwai & Mishkin, 1969). The types of learning required in these tasks are different since they are sensitive to lesions in different brain areas and the visual pair-association task was more sensitive to the medial temporal lesion (Iwai & Mishkin, 1969; Murray et al., 1993; Suzuki et al., 1993). This fact prompted us to test the roles of IEGs in the consolidation process of cognitive memories in primates. We found selective expression of zif268 only during the learning of the pair-association task.

**Methods of Experiment 2**

Animals and visual memory tasks. The subjects were six male macaque monkeys (M. fuscata), all weighing between 7.0 and 9.6 kg at the time of perfusion. Three monkeys were trained to learn a visual pair-association task and the others were trained to learn a visual discrimination task. A set of 12 pairs of the Fourier descriptors was used as a visual stimulus set in both pair-association and visual discrimination tasks. The procedure of the visual pair-association task was essentially the same as described under Experiment 1 except that monkeys sequentially learned the training set and the test set of paired associates in this study. In the other task, the visual discrimination task, monkeys were required to touch the rewarded stimulus to obtain fruit juice. The monkeys also sequentially learned the training and test sets of visual stimuli in this task.

The monkeys were first trained to perform the tasks with a set of stimulus pictures (training set). After the monkeys had learned the task rule with the training set, a new set of stimuli (test set) was introduced. The monkeys executed approximately 600 trials in a training session each day in both the pair-association task and the discrimination task after the introduction of the test set. On the 8th to 10th day with the test set, the monkeys learning the pair-association task were anesthetized and immediately perfused following that day's training session as described below. The monkeys learning the discrimination task were perfused after the training session on the 5th or 6th day. The monkeys in both groups were exposed to an equivalent number of visual stimuli (pair-association, 684 ± 102 stimuli per hour; discrimination, 664 ± 72 stimuli per hour) during the 2 h before the perfusion.

Tissue preparation, immunohistochemistry, and antibodies. After the training session on the perfusion day, the monkeys were immediately given an overdose of pentobarbital (>40 mg/kg) and then perfused transcardially. The brain blocks were frozen in dry-ice powder, and sections (32 μm) were cut using a cryostat and stained immunohistochemically as described previously (Okuno et al., 1995). In brief, after being washed with PBS, the sections were incubated with blocking solution containing PBS plus 10% normal goat serum and 0.3% Triton X-100 for 60 min at room temperature, followed by incubation with blocking buffer containing the primary antibody (see below) at 4°C for 24...
h and then by incubation with blocking buffer containing horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (Jackson ImmunoResearch; Catalog No. 111-035-003; diluted 1:400) at room temperature for 2 h. After being washed, the sections were reacted with coloring solution (0.2 mg/ml 3,3'-diaminobenzidine tetrahydrochloride, 0.05% H₂O₂, 0.08% nickel chloride in 50 mM Tris–Cl, pH 7.4) for 10 min at room temperature.

All primary antibodies used in this study were rabbit polyclonal antibodies. An anti-Zif268 antibody was raised against a synthetic oligopeptide (19 amino acid residues) corresponding to the carboxy terminus of Zif268. The antigenic sequence is evolutionally conserved among various species, including rodents and humans. The specificity and reactivity of the anti-Zif268 antibody to the monkey zif268 gene product were determined previously (Okuno et al., 1995); the anti-Zif268 antibody specifically recognized an 86-kDa protein in the nuclear extracts from monkey cerebral cortex in immunoprecipitation experiments. An anti-c-Fos antibody was purchased from Oncogene Science Inc. (Catalog No. PC05).

Image analysis. To visualize the spatial distribution of Zif268, an image (resolution 4096 × 4096 pixels for 1.57 × 10² mm²) was obtained from each of the serial Zif268-immunostained sections at 0.5-mm intervals (20 sections for each subject) by a CCD camera attached to a microscope, and optical density (OD) was measured in each pixel (pixel OD) using a computer-aided image analyzer (IBAS-V2.0, Zeiss).

The cortical area (layers II to VI) in each section was segmented into 0.5-mm-wide strips radially from the white matter to the cortical layer I (see Fig. 4A, left). In each segment, the pixel ODs could be classified into two groups, pixel ODs originating from Zif268-immunostaining reaction products in the nuclei and those originating from background staining. The pixel ODs originating from background staining were estimated by fitting a Gaussian distribution to the lowest peak of the distribution of pixel OD using the maximum-likelihood method. The density of Zif268-immunostaining reaction products in each segment was calculated by summing all pixel ODs after subtracting ODs originating from background staining and by normalizing to the area of the segment. The segments were reconstructed into a straight-line, unfolded, two-dimensional map (see Fig. 4A, right), essentially according to the procedures described by Van Essen and Maunsell (1980). The density of the immunostaining reaction products is indicated on the unfolded map in pseudocolor representation.

Results of Experiment 2

To investigate the formation of associative memory of the visual stimuli but not skill-based or habit-like memory incidental to the task paradigm, we first trained the monkeys to learn a rule or strategy of the tasks, which is considered to be related to the latter memory classes, using a set of 24 pictures (training set; see Methods). After the monkey's performance reached a plateau level with the training set, a new stimulus set (test set) was introduced to access formation of the new associative memory of the new visual stimuli. The monkey's performance was at a chance level (i.e., 50% correct responses) in the first session with the test set and then improved in subsequent test sessions.
The monkeys were perfused immediately after the completion of the test session before the performance reached a plateau phase.

We first examined the expression of Zif268 in the anterior temporal cortex of the monkeys during visual paired associate learning and visual discrimination.
learning. During visual paired associate learning, intensely Zif268-immunopositive neurons were observed in the inferior temporal gyrus, the area that lies between the rhinal sulcus and the anterior middle temporal sulcus; the intensely Zif268-immunopositive neurons accumulated in patches in the ventral surface of the inferior temporal gyrus rather than in the banks of the rhinal sulcus or the anterior middle temporal sulcus. The patches were centered in layer IV and spread into both superficial (II/III) and deep (V and VI) layers. The patchy pattern was found in several consecutive sections and was specific to Zif268 since other IEG products (cFos and J uD) did not show such patterns in the adjacent sections.

The distribution of Zif268 expression in the monkey temporal cortex was visualized by image analysis and displayed on a two-dimensional unfolded map (Fig. 4). In each monkey with visual paired associate learning, Zif268 was expressed at high levels in a strip parallel to the rhinal sulcus in an anterior–posterior axis, particularly at several spots in this strip (PA1–PA3). Zif268 was expressed at relatively low levels and was distributed more homogeneously in the monkeys with visual discrimination learning (VD1–VD3). The inferior temporal gyrus is composed of three cytoarchitectonically and connectionally distinct areas (area 35, area 36, and the ventral part of area TE) (Suzuki and Amaral, 1994). The expression of Zif268 in the monkeys during visual paired associate learning was prominent in area 36. By contrast, in area 35, which is medially adjacent to area 36, Zif268 expression levels were low during both types of visual learning. A two-way ANOVA for the mean expression level (two learning tasks and three brain areas) revealed significant effects of task [F(1, 4) = 12.05, p = .025] and of area [F(2, 8) = 17.73, p = .001]. The expression in area 36 was significantly higher during visual paired associate learning than during visual discrimination learning [1.34 ± 0.07 and 0.96 ± 0.04 (±SEM, arbitrary units), respectively; p = .01]. In area 35, the expression levels in the two monkey groups were similar and were not significantly different (pair-association, 0.65 ± 0.09; discrimination, 0.58 ± 0.05; p > .5). In ventral TE, the difference did not reach statistical significance (p = .22), but there might be a tendency for higher expression of Zif268 in the monkeys with visual paired associate learning (pair-association, 1.11 ± 0.15; discrimination, 0.85 ± 0.08).

GENERAL DISCUSSION

In this article, we examined the neural mechanisms underlying the memory consolidation process by single-unit recording and molecular biological methods during a visual pair-association task in monkeys. In Experiment 1, we tested the role of the backward signal from the medial temporal lobe to the IT cortex. We made lesions in the entorhinal and perirhinal cortices and recorded unit activity in the IT cortex. In Experiment 2, we investigated the molecular basis of circuit reorganization by detecting the expression of immediate-early genes in monkey temporal cortex. The results of Experiment 1 and Experiment 2 supported the hypothesis that limbic–neocortical interactions are critical in the consolidation process since limbic neurons undergo rapid modification of synaptic connectivity and provide backward signals that guide the reorganization of neocortical neural circuits.

In Experiment 1, the results provided new evidence about the role of the
backward signal. The pair-association learning task has been used to test declarative memory in humans, and the role of the medial temporal lobe in declarative memory formation was proposed on the basis of neuropsychological and behavioral evidence. The present experiment evaluated single neuron responses for visual pair-association and supported the view that the IT neurons have the ability to represent the long-term mnemonic code between picture pairs and that the ability is critically dependent on backward signal from the entorhinal and perirhinal cortices to the IT neurons.

In this experiment, we combined single-unit recording techniques with surgical manipulations in chronic monkey preparations; this chronic monkey preparation was designed so that a single IT neuron becomes devoid of limbic backward signal but can receive forward visual signal in a normal behavioral context (Fig. 2). It was demonstrated by a lesion study that monkeys with bilateral removal of the entorhinal and perirhinal cortices neither relearn visual pair-association that was learned prelesionally nor learn new associations among new visual stimuli (Murray et al., 1993). In the present experiment monkeys with a unilateral lesion learned both the old and the new associations easily. The milder effects of the unilateral lesion at the behavioral level should be due to the intact temporal lobe in the contralateral hemisphere (Fig. 2). At the level of neuronal response, the lesion wiped out the ability for association of the IT neurons that had been isolated from the intact temporal lobe in the contralateral hemisphere by the anterior commissurectomy.

In the primate inferior temporal cortex and part of the superior temporal cortex, neurons selectively respond to complex objects such as faces, hands, Fourier descriptors, and fractal patterns (Miyashita, 1993). Several lines of experimental evidence showed that some, if not all, of these responses were acquired through learning in adulthood (Miyashita, 1993; Higuchi & Miyashita, 1996). The present results show that the backward connection from the limbic cortex is indispensable for some kinds of these acquired responses, such as pair-association coding, but may not be indispensable for others. Indeed, the fractal patterns introduced after the lesion (set B) could activate IT neurons highly selectively, which suggests preservation of the tuning mechanism that molds and sharpens neuronal response selectivity to an object through repetitive stimulus presentation (Sakai, Naya, & Miyashita, 1994). These results give concrete evidence for the decomposition of the primate long-term memory system into several different neural mechanisms.

In Experiment 2, we detected the altered expression of Zif268 in the monkey inferior temporal gyrus, especially in area 36, during visual paired associate learning compared with that during visual discrimination learning. During visual paired associate learning, Zif268-immunopositive neurons accumulated in patches that spread into the superficial and deep layers in the inferior temporal gyrus. High-level expression of Zif268 was localized in a strip along the rhinal sulcus (Fig. 4B). In contrast to the strong patchy expression of Zif268, the expression of other IEG products (Fos and JunD) did not show such patterns. The results indicating that Zif268 expression was most evident in area 36 during paired associate learning are consistent with the results of Experiment 1.

In Experiment 2, we used visual discrimination learning as a control for visual paired associate learning because, under the no task condition, it was difficult to regulate mental activity related to memory formation or even to regulate
motor activity in the monkeys. The difference in Zif268 expression level between visual paired associate learning and visual discrimination learning most likely originated from differences in neuronal activities related to memory requirements for the two tasks, for several reasons. First, the experimental conditions were almost identical for the two tasks; i.e., the same task apparatus with the same touch sensor, as well as the same visual stimuli and the same reward, were used, suggesting that the differential expression of Zif268 was not related to general motor or sensory activity. Second, the difference was not associated with the amount of sensory input; i.e., the number of trials in the session immediately before the perfusion was not related to the Zif268 expression level in any of the three brain areas in the inferior temporal gyrus. Third, Zif268 expression levels in area 35 or in the hippocampus were almost the same for the two monkey groups, confirming the reproducibility of immunostaining among different sections and in different monkeys.

Although it is not yet well characterized in the mammalian brain, studies in both invertebrates and vertebrates have revealed that formation of long-term memory requires new protein and mRNA synthesis whereas formation of short-term memory does not (Bailey & Kandel, 1993). The requirement of de novo protein and mRNA synthesis for long-term memory suggests that neuronal activities induced by learning initiate a cascade of gene expression. The first step of the gene cascade is thought to be the expression of IEGs. Transcription factors encoded by IEGs play a role in the establishment of long-term changes in the properties of synapses (Morgan & Curran, 1991; Abraham et al., 1993). The induced transcription factors regulate expression of late-response genes that probably contribute to synaptic plasticity, especially morphological changes. For example, synapsin-I and neurofilament-light genes are activated by Zif268 (Thiel et al., 1994). Moreover, many IEGs including zif268 have cyclic AMP-responsive element (CRE) sequences in their promoter regions and their expression can be controlled by CRE-binding proteins (CREBs) (Vaccarino et al., 1993). Recent studies have shown CREB involvement in the formation and consolidation of various classes of long-term memory in several species (Bourtchuladze et al., 1994; Yin et al., 1994). Taken together, the expression of Zif268 in the temporal cortex observed in this study suggests that Zif268 may participate in the gene cascade related to formation of visual associative memory in primates.

REFERENCES


