THE NEURONAL BASIS OF VISUAL MEMORY AND IMAGERY IN THE PRIMATE: A NEUROPHYSIOLOGICAL APPROACH

KIYOSHI NAKAHARA,*1 MACHIKO OHBAYASHI,*2 HYOE TOMITA,*2 AND YASUSHI MIYASHITA*1,*2

*1Mind Articulation Project, ICORP, JST, Bunkyo-ku, Tokyo 113-0034 and *2Department of Physiology, University of Tokyo, School of Medicine, Bunkyo-ku, Tokyo 113-0033, Japan

“The Astonishing Hypothesis is that ‘You,’ your joys and your sorrows, your memories and your ambitions, your sense of personal identity and free will, are in fact no more than the behavior of a vast assembly of nerve cells and their associated molecules.”

Francis Crick (1)

Experience changes the behavior of an animal by modifying its nervous system. We refer to such modification as learning and, in the classical view, to the retention of such modification as memory. Since the appearance of cognitive psychology in the late 1950s and 60s, memory has been considered to include memorization, retention, and recall. Our lives are, after all, accumulations of individual memories.

To understand the biological basis of memory is one of the
most challenging frontiers of science. To accomplish this, we must determine what happens in the nervous system when a particular memory is acquired. In other words, we need to know neuronal correlates of memory. Recent advances in neuroscientific methods have enabled us to investigate the mechanisms of brain functions in detail. In particular, molecular biological methods, such as gene targeting, and noninvasive brain imaging methods, such as those using functional magnetic resonance imaging (MRI), are powerful tools for investigating brain functions. However, if we want to explore the neuronal correlates of memory at a system level, from the activity of a single cell to those of cell networks, use of a "single unit recording" technique will be most effective.

I. SINGLE UNIT RECORDING

Single unit recording is a technique for recording action potentials of single neurons from both anesthetized and awake animals. Briefly, a small part of the skull is removed under anesthesia, and then a fine electrode consisting of an insulated wire with a tiny exposed tip is inserted into the brain. Such a microelectrode can record action potential of a single neuron, from outside the cell, if the tip of the electrode is very close to that cell. By moving the tip of the electrode along its length from one place to another in the brain, it can detect the action potentials of one neuron after another from outside the cells. Currently, sets of more than one electrode are often used, so that the action potentials of many neurons can be recorded simultaneously. Here, we employ this technique for exploring the neuronal correlates of the visual associative memory in the primate brain. Primates are used both because they are sufficiently clever to learn complex memory tasks, and because their visual system is highly similar to that of humans.

Experimenters can select where they put their electrodes into the monkey's brain, but exactly which type of neuron they record from is somewhat a matter of chance. Thus it is quite important to select the proper recording site to achieve good results. Based on the anatomical and neuropsychological evidence presented in the next three sections, we hypothesized that the inferior temporal cortex (IT) is one of the best candidates for exploring the neuronal correlates of visual memory in both humans and primates.
II. OUTLINE OF THE VISUAL PATHWAY

The association cortex refers to cerebral cortical regions other than primary motor and sensory areas, and is remarkably large and well-developed in primates and other higher animals. In such animals, objects are recognized visually by circuits of neurons in the visual association cortex. Visual information from the retina reaches the striate cortex (primary visual cortex, V1) after being relayed through the lateral geniculate nucleus (Fig. 1). Within the striate cortex, individual modules of neurons analyze information from restricted regions of the visual scene that pertain to movement, orientation, color, binocular disparity, and spatial frequency. Information about each of these attributes is collected in the prestriate cortex, a parasensory association cortex that surrounds the striate cortex (Fig. 1). The prestriate cortex consists of some hierarchical subregions in the primate (2). For example, specific regions are devoted to the analysis of form, color, and movement. At this stage, visual information is divided and sent along one of two pathways (3) (Fig. 1). One pathway turns upward, ending in the posterior parietal cortex. This dorsal pathway recognizes where the object is located. Another pathway turns downward, ending in the IT. This

![Diagram of the visual pathways in the monkey's brain](image)

*Fig. 1.* Lateral view and two visual pathways of the monkey's brain. Lateral view of the monkey's brain (right hemisphere) and the major divisions of the visual cortex are shown. The arrows indicate the two visual pathways.
ventral pathway recognizes what the object is. Thus the IT is the final stage for recognizing the shapes of objects. In the next section, we will discuss the neural connections of the IT in detail.

III. NEURAL CONNECTIONS OF THE IT

The IT receives visual input from areas V4, V4t, DP, and VOT in the prestriate cortex, from areas TF and TH on the parahippocampal gyrus, from area TG at the temporal pole, from the FST at the fundus, and from the STP at the dorsal bank of the superior temporal sulcus, as well as massive connections within the cortex (2, 4, 5). Among these sources, that of area V4 has been considered to provide the major source of retinal information. However, IT neurons have been shown to clearly respond to visual stimuli after lesion of V4, although their responses were weaker and less discriminative than normal (6). Relative contributions from other inputs have not yet been quantitatively examined.

The IT, in turn, projects to the limbic system, including the amygdaloid nuclei (7, 8) and the hippocampus via the entorhinal cortex (9, 10). The IT also projects to areas 8, 12, and 45 in the frontal cortex (11, 12) (Fig. 2).

The anatomical position of IT is thus referred to as the final

![Fig. 2. Connections and functional properties of the IT and other cortices.](image-url)
link between the visual cortices and the limbic systems and frontal lobe (Fig. 2).

IV. NEUROPSYCHOLOGICAL EVIDENCE

Many clinical and lesion studies have shown that damage of the IT disrupts the ability to discriminate among different visual stimuli. Patients who have undergone a right anterior temporal lobectomy have been shown to be mildly impaired in perceptual tasks in which the normal redundancy of the stimuli have been reduced, such as in Mooney’s Closure Face Test (13). These patients are also markedly impaired in recognition memory for complex visual patterns that cannot be easily coded by words (such as faces and irregular abstract designs) (14, 15). These deficits are unrelated to the extent of hippocampal removal in the temporal lobectomy (13, 16). Thus the temporal neocortex is itself critically involved in such representational memory. This result closely parallels the well-established findings in monkeys that bilateral excision of the anterior inferior temporal cortex (AIT) produces severe and lasting deficits in visual object recognition (17). The anterior temporal lesions also impair visual pattern discrimination, but more posterior, temporo-occipital lesions produce the most severe perceptual deficits in the monkey (18, 19).

Two attributes further characterize the role of this cortex. First, the AIT has been suggested to play a role in storing the “prototype” of a visual object (20, 21). Earlier research showed impairments in size constancy in monkeys with lesions of the IT (22, 23). More recently, lesions of this cortex have been shown to impair the discrimination of objects transformed by size, orientation, or shadow configuration after learning in an untransformed mode (21). The concept of “object-centered representation” is a counterpart in the computational approach (24). Single-unit recording data support the hypothesis, as will be shown in later sections.

Second, there is now compelling evidence that the temporal neocortex contributes to the visual recognition process differently than the limbic cortex does (reviewed by Miyashita (25), and Squire and Zola-Morgan (26)). The distinction can best be interpreted in terms of the hypothesis that memory has at least two
components. One is the recently acquired, labile memory that can be readily disrupted by a head injury, as retrograde amnesia demonstrates clinically (27). The other is the remote, fully consolidated memory (28). Drug applications selectively depress or facilitate the labile component (29).

Bilateral damage to the medial temporal region, which includes the hippocampus, amygdala, and adjacent cortex (30), or to only the hippocampal CA1 field (31), has been shown to accompany limited, short-span retrograde amnesia in humans. In monkeys, the effects of hippocampal lesions (including those to the entorhinal and parahippocampal cortex) on the retention of 100-object discrimination problems have been measured (32). The monkeys were severely impaired at remembering recently learned objects, but could remember objects learned long ago as well as normal monkeys. These data suggest that the labile component of the long-memory is localized in the hippocampus and adjacent cortex. Presumably, a later and more consolidated stage of long-term memory is represented in AIT (25), that is, the AIT not only combines attributes to produce a particular visual image, but also works as a visual memory storehouse. Thus AIT is a good target for investigating the neuronal mechanism of long-term visual memory.

V. VISUAL MEMORY TASKS

We decided to record the responses of single neurons in primate AIT in order to investigate the neuronal correlates of visual memory. Here, an important point was the design of the visual memory task that monkeys were required to learn and perform during recording. As shown by Miyashita (25), one promising strategy is to have monkeys memorize an artificial associative relation among pictures, and then to examine whether the picture-selective activities of temporal cortical neurons reflect the stimulus-stimulus associations imposed during learning. If the artificial associative relation among pictures tends to affect the stimulus selectivity of neurons, then the neural selectivity is acquired through learning and represents neuronal correlates of the associative long-term memory of the pictures.

There are two major types of visual memory tasks: delayed matching-to-sample task and pair-association task. The delayed
matching-to-sample (DMS) task requires that the animal remember a particular stimulus for a period of time. Typically, a cue picture is presented first. After a delay period, pictures are presented and then the subject is required to choose one matching the cue picture. This task is thus mainly concerned with short-term memory. However, a fixed-order presentation of the pictures during the training session affected the selectivity of neuronal responses to pictures (33). This case shows that long-term memory can modify the neuronal response selectivity to pictures during the DMS task. On the other hand, the visual pair-association (PA) task requires the animals to memorize artificial associative pairs between pictures (34), and is thus directly concerned with long-term memory.

Lesion studies have demonstrated that monkeys whose medial temporal region had been removed bilaterally could learn neither the DMS task (17, 26, 35) nor the PA task (34). The type of memory these tasks employed would, therefore, correspond to one that relies on the integrity of these structures.

To investigate the neuronal correlates of long-term visual memory, we performed single unit recordings from the AIT of monkeys while they were performing the PA task. The 24 stimuli
were generated with a computer, and the 12 pairings were made randomly. As can be seen in Fig. 3, the members of pairs do not particularly resemble each other. The task was as follows: the monkeys were shown one member of the pair (cue stimulus) presented on a video screen for 1 sec. Then, after a delay period of 4 sec, they were shown two choice stimuli: the paired associate of the cue and a stimulus from other pairs (distracter). If the animal touched the correct stimulus on the screen within 1.2 sec, he received a drop of grape juice. This task paradigm reliably assured the learning of visual stimuli, since the monkeys could not select paired associates correctly without memorizing and recalling pair combinations.

VI. PAIR-CODING NEURON

A few months into the training term, picture-selective neuronal responses were found in the AIT during the cue period (36). Many neurons responded reproducibly to only a few pictures. And neurons responding to one member of a pair were likely to respond to the other member as well. For example, the neuron having the activity shown in Fig. 4 responded to only two stimuli making a pair: stimuli 6 and 6'. If a particular neuron responds to only two of twenty-four different stimuli, the likelihood of these two stimuli being members of the same pair is only one in twenty-three. The cells might have responded to geometrically similar patterns, but in many cases, the strongest and second strongest responses were as-

![Fig. 4. Responses of a pair-coding neuron. Typical responses of pair-coding neuron that exhibited form-selective activity during the cue period. (A) Trials for cue 6' that elicited the strongest cue response. (B) Trials for cue 6 that elicited the second strongest cue response.](image-url)
cried to a particular pair that had no apparent geometrical similarity. Some other cells showed broader tuning and responded to more than three pictures. Nevertheless, paired pictures were among the most effective stimuli for these cells. We termed this type of cell a "pair-coding neuron," that manifests selective cue responses to both pictures of the paired associates. These responsive cells tended to be located in close proximity (1–2 mm in width).

The association property was analyzed by calculating two coupling indices for each neuron as follows:

\[ CI_p = \frac{1}{N_p} \sum_{i \neq j, j \neq i^{'}} \frac{(x_i - b)(x_j - b)}{(x_{\text{best}} - b)(x_{2\text{nd-best}} - b)} \times 100, \]

with \( j = i^{'} \) for paired associates,

\[ CI_r = \frac{1}{N_r} \sum_{i \neq j, j \neq i^{'}} \frac{(x_i - b)(x_j - b)}{(x_{\text{best}} - b)(x_{2\text{nd-best}} - b)} \times 100, \]

with \( j \neq i^{'} \) for random combination

where \( x_i \) denotes a mean discharge rate during the cue period for the \( i^{th} \) picture (the \( i^{th} \) and \( i^{'} \)th pictures are paired associates), \( b \) is a spontaneous background discharge rate, \( x_{\text{best}} \) and \( x_{2\text{nd-best}} \) are mean discharge rates for the best and second-best cue-optimal pictures in each cell, and \( N_p \) and \( N_r \) are the total number of combinations for two cases.

One coupling index (denoted as \( CI_p \)) measures correlated neuronal responses to paired associates, whereas the other coupling index (\( CI_r \)) estimates responses to other random combinations among the 24 pictures. The latter index, \( CI_r \), serves as an experimental control for untrained association between two pictures. For each cell, a pair index (\( PI \)) was defined as \( (CI_p - CI_r) \). Analysis of the frequency distribution of \( PI \) values demonstrated that the paired associates elicited significantly correlated responses. It was therefore hypothesized that the selectivity of these neurons was acquired through learning of the pair-association task. Although the cellular mechanisms for these phenomena are not yet clear, a possible basis for the memory coding lies in the change of synaptic connections through repetitive learning.
VII. PAIR-RECALL NEURONS

Electric stimulation of the temporal lobe induces visual experiential response and hallucination in humans (37). Penfield hypothesized that a particular anatomical site was related to specific, repeatable imagery, and thus to a specific "memory record". However, although many researchers have confirmed evoked complex hallucinations, this hypothesis remains controversial (38). No other clues to the memory retrieval process have been reported.

In the PA task, we observed another interesting result. We found that many neurons began to respond during the delay period, as if they were part of a circuit that recalled the choice picture (36)

Fig. 5. Responses of pair-recall neuron which exhibited form-selective activity during the delay period. (A) Trial for cue 12 that elicited the strongest cue response. (B) Trials for cue 12' that elicited the delay response, presumably reflecting recall of the pair associate 12. Note the tonic increasing activity during the delay period, which is much stronger than the cue response. (C) Trials for cue 1 that resulted in the second-strongest cue responses. (D) Trials for cue 1'. Note the sustained delay activity and the inhibitory cue response. Trials for cue 3 (E) and for cue 3' (F) elicited no response.
(Fig. 5). In this case, picture 12 elicited the strongest response from a single neuron during the cue period (Fig. 5A). When picture 12', the paired associate of this optimal picture 12, was used as a cue, the same cell exhibited the highest tonic activity during the delay period, in contrast to a weak response during the cue period (Fig. 5B). 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. 5C, D). Other pictures evoked weak or no response (Fig. 5E, F). The delay activities were confined to a few cue stimuli in the set. This type of cell was called a “pair-recall neuron”, in which the paired associates of a cue-optimal picture elicited the highest delay activity. The delay activity of the pair-recall neuron is not a mere sensory after-discharge, because it is always stronger than the cue response. Significant augmentation of the discharge rates was observed for the highest delay activity, whereas delay activity elicited by a cue-optimal picture itself was significantly reduced during the delay period.

Anticipatory neural activities that precede the initiation of motor response and that increase during the preparatory period have been reported in the primate frontal cortex (39, 40). In the PA task, the increasing delay activity of pair-recall neurons is not related to motor response, because the monkeys could not predict which position should be touched. As noted above, this delay activity is not only picture-selective, but also closely coupled with the paired associate that is not actually seen but recalled. So this delay activity may reflect the neuronal correlates of our conscious imagery.

VIII. THE PACS TASK

To further investigate these neural mechanisms of visual imagery, we developed a novel task, the pair-association with color switch (PACS) task, which combined the PA and DMS tasks (41). The PACS task consisted of two patterns of trials, the PACS trial and the DMS trial. Both kinds of trials were arranged at random. In the PACS trial, the necessity for image generation and its time were controlled by switching color of the fixation square in the middle of the delay period. In the DMS trial, on the other hand, there was no
Fig. 6. The PACS task. (A) Sequence of events in the PACS trial or the DMS trial. Cue stimuli and squares were presented at the center of a video monitor. Choice stimuli were presented randomly in two of four positions on the monitor. Warning, gray square (1 sec in both trials); cue, 1 of 24 pictures in B as a cue stimulus (0.5 sec); delay period 1, square that has the same color as the cue picture (3 sec in the PACS trial); delay period 3, gray square (1 sec in both trials); choice, a choice of two stimuli (1.2 sec in both trials), the paired associate of the cue (correct) and one from a different pair (error) in the PACS trials, or the same picture as the cue (correct) and one from a different pair (error) in the DMS trials. (B) Twelve pairs of pictures used as stimuli in both trials. The first pair is picture G1 (green) and picture B1 (blue), the second pair is G2 and B2, etc.
color switch and the monkey chose the same picture as a cue. In both trials, the same set of 12 pairs of pictures was used as visual stimuli, with each pair consisting of a green picture and a blue picture (Fig. 6B). The sequence of events in these trials was as follows (Fig. 6A). When the monkey pressed a lever in front of the screen, a white square (warning) was presented at the center of the

Fig. 7. Differential delay responses of a single A1T neuron in the PACS task. (a–d) Responses in the PACS trial. (e–h) Responses in the DMS trial. (a and e) Responses in trials with cue G7. (b and f) Responses in trials with cue B7. (c and g) Responses in trials where one picture (g) of G1-G12 except G7 was used as a cue. (d and h) Responses in trials where one picture B of B1-B12 except B7 was used as a cue. Picture G7 elicited the strongest cue response in both trials (a and e). Note the suppressed response during delay period 2 (d2) and delay period 3 (d3) in the PACS trial (a) but not in the DMS trial (e). We called this phenomenon the pair-suppression effect. In trials with cue B7, little response was observed during the cue period in both trials (b and f). Note the enhanced response during delay period 2 and delay period 3 in the PACS trial (b) but not in the DMS trial (f). We termed this the pair-recall effect. In trials with cue G or B, no response was observed in either trial (c, d, g, and h), indicating that there was no significant color effect.
screen for 1 sec. Following the cue presentation of one of the 24 pictures for 0.5 sec, there was a delay period during which a square was presented. Color of the square was the same as that of the cue during the first delay period (delay period 1: 2 sec in the PACS trial; 5 sec in the DMS trial). In the PACS trial, the color of the square changed to that of the paired associate after delay period 1, signaling the initiation of retrieval, and the second part of the delay period (delay period 2: 3 sec in the PACS trial; not included in the DMS trial) commenced. To balance the visual stimulus conditions in the two trials, a gray square was presented for 1 sec during the third delay period (delay period 3: 1 sec in both trials). After delay period 3, a choice of two stimuli was shown randomly in two of four possible positions (arranged in two rows of two columns). In the PACS trial, the choice stimuli were the paired associate of the cue (correct) and a distracter (error), and in the DMS trial, they were the same picture as the cue (correct) and a distracter (error). Thus, the monkeys did not know which trial pattern was being used until delay period 2. At that time, they had to choose a paired associate (PACS trial) or the same picture of the cue (DMS trial) by referring to the color switch. We recorded activities of single neurons in the IT during the performance of this task.

Figure 7 shows a set of typical responses. One picture (G7, Fig. 6B) elicited the strongest response during the cue period (Fig. 7a; here, we call this cue optimal picture the best picture, irrespective of its delay response). The excitatory response was maintained in delay period 1. The paired associates (B7) of the best picture (G7) elicited little response during the cue period and delay period 1 (Fig. 7b). However, this neuron started to respond just after the onset of delay period 2, when the color of square changed from that of the cue (B7) to that of the paired associate (G7). The picture-selective activation after the color switch in the PACS task was termed the “pair-recall” effect. The pair-recall effect continued from delay period 2 into delay period 3, in which the color of the square was the same gray in both trials (Fig. 6A). Thus, the pair-recall effect was not due to the square’s color. This was also confirmed by the fact that the pair-recall effect was observed in trials with cue B7 but not in trials where other blue pictures were used as a cue. In the DMS trial, during the cue period and the delay period 1, the response selectivity was the same as that of the PACS trial.
(Fig. 7e). However, in the DMS trial there was no significant response during the delay period (Fig. 7f). These results suggest that this delay discharge, which was specific to the PACS trial, was triggered by image generation in the mind of the monkey.

IX. CONCLUSIONS AND FUTURE PERSPECTIVES

In this review we introduced anatomical, neuropsychological, and neurophysiological approaches for investigating the mechanisms of memory. We also presented some results of experiments that identified specific populations of neurons that play an important role in visual memory storage and recall in the IT cortex of primates. In this final section, we will point out some further questions and problems.

First, the question of how objects are encoded in single cells merits further investigation. If populations of neurons represent particular objects, how many neurons are required to represent one object? Are there any temporal or spatial correlations between these neurons? Another challenging question is whether or not microstimulation or selective pharmacological inactivation of these neurons can affect the behavior of monkeys.

Second, cortical memory is likely to be consolidated over time under the influence of the afferents from the medial temporal region (Fig. 2). Investigation into the time course of this process in single cortical neurons would make a suitable subject for future research.

Third, we have now obtained a first clue to the memory recall mechanism at the single-cell level. Although it is uncertain how or from where the recall-related activity in the pair-recall neurons originates, these questions would be experimentally accessible. Dissection of the uncinate fasciculus, the association fibers reciprocally connecting the IT and the prefrontal cortex (PFC), results in deficit in performance of the PA task (42). Thus, interactions between the IT and PFC may be a source of such recall-related activity (Fig. 2).

Fourth, because the brain is very complex, we will need to investigate it at every level, from the molecular to the computational level. At the same time, it will be necessary to integrate different levels of research. To date, however, attempts at such integration have been insufficient. Neurophysiological and behavioral
analyses of gene-targeted mice are useful to correlate the function of particular molecules with higher neuronal functions. However, such methods are still too rough to investigate these correlations precisely. Tsien et al. (43) used the Cre/loxP recombination system to delete the NMDA receptor function specifically in the hippocampal CA1 region. This conditional gene targeting technique may provide a way to link molecules, synaptic plasticity, and behavior.

To understand the brain functions is to understand ourselves. Although we are still in the early stages of exploring the mechanisms of brain functions, we will one day be able to clarify these — as long as we believe in the power of science.

SUMMARY

To understand the biological basis of memory is one of the most exciting frontiers of science. Single unit recording is a powerful method to investigate neuronal correlates of various brain functions such as memory in awake animals. Anatomical, neuropsychological, and neurophysiological evidence indicates that the IT has an important role not only for synthesizing the analyzed visual attribute into a unique configuration, but also for the storehouse of visual memory in humans and primates. We performed single unit recordings in the primate IT, and found neuronal correlates of visual long-term memory: the IT neurons could reflect learned associative relations among stimuli. The findings reviewed here support the hypothesis that the IT is a region of the brain where visual perception meets memory and imagery.

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