other four pairs into grade 1. None of the pairs with differences in optimal orientation of more than 46° (grades 3 and 4) manifested any inhibitory interaction. That is, inhibitory interactions were found mostly in the cell pairs with slightly different or rather similar orientation preferences. There were no notable differences in the magnitude of the inhibition between the cell pairs of grade 2 and those of grade 1. The mean horizontal distances between the pairs of cells in grades 1 and 2 were 231 ± 125 and 362 ± 241 μm respectively. As to the laminar location of the cells, nine pairs with inhibitory interactions were in the same layer and the remaining one was in a neighbouring layer (Table 1). Half of the pairs having inhibitory interactions were located in cortical layer IV.

The present results demonstrate that horizontal inhibitory interactions exist between visual cortical neurons having somewhat different orientation preferences, but appear not to exist between those displaying orthogonal preferences. This conclusion provides evidence differing from previous conflicting results[8-11]. The negative conclusion of Ts'o *et al.*[9] on the existence of horizontal inhibitory interactions may be due to their restriction of sampling to cells from layers II/III of the cortex. In these layers, their samplings may have been biased strongly towards recordings from pyramidal cells, which are believed to be connected by excitatory synapses[10,11]. In fact, previous studies using the cross-correlation technique have been able to detect inhibitory interactions in visual cortex, although they have been found mainly in a vertical (that is, intracolumnar) direction[21,22]. It has been reported, however, that cross-correlation analysis is a technique that can be relatively insensitive to inhibitory interactions[23]. Accordingly, we cannot completely exclude a possibility that we may have underestimated the extent of the existence of horizontal inhibitory interactions. In this sense, our findings are not altogether inconsistent with the suggestion of Matsubara *et al.*[8,24] that patchy horizontal connections exist between cells with orthogonal orientation preferences and that these connections may inhibit each other. It should be pointed out, however, that the functional effectiveness of such connections may be insufficiently strong to be detectable by the present methods, even if they exist. So, it seems reasonable to propose that cross-orientation inhibition effectively operates for cell-cell interactions between groups displaying similar orientation preferences to quite dissimilar but not orthogonally opposed preferences.

This conclusion is consistent with anatomical evidence. Several types of visual cortical neurons that are thought to be inhibitory have axons extending horizontally by about 1 mm in length, in addition to having major vertical projections[25]. For example, a type of small basket cell called the 'clutch cell' has horizontal axonal projections extending 300–500 μm in layer IV (ref. 26), in which most horizontal inhibitory interactions have been found in the present study. Also, the observation that horizontal projections of axons of inhibitory neurons are limited to the same layer or to neighbouring layers[25] is consistent with the present findings.

These results support data from previous psychophysical experiments suggesting that there is a mutual inhibition between orientation detectors with slightly different preferences[27], and are consistent with physiological experiments indicating that inhibitory interactions are tuned broadly to the same orientation as excitatory inputs so as to sharpen the orientation tuning of cortical cells[3,28,29]. This conclusion seems at odds with the intracellular records of Ferster[7] indicating that i.p.s.ps of cortical cells are tuned to orientations as sharply as excitatory postsynaptic potentials. There is the possibility, however, that inhibitory synapses may be distantly located from the soma from which his recordings were made, or additionally, a 'shunting inhibition' without a sizable hyperpolarization might have a role in selectivity. Although there are data arguing against this latter possibility[30], presynaptic inhibition might also be involved in helping to make cortical cells selective in their responsiveness.

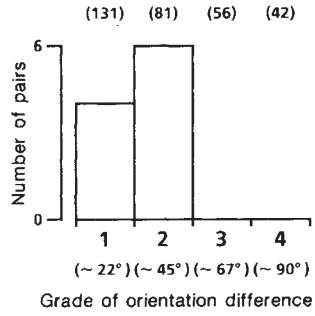

Fig. 2 Histogram displaying the number of pairs with inhibitory interaction falling in each of four optimal orientation-difference groups. Each group represents pairs of cells having orientation differences: 1, between 0–22°; 2, 23–45°; 3, 46–67°; 4, 68–90°. Total number of pairs sampled in each group is indicated at the top.

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1. Hubel, D. H. & Wiesel, T. N. *J. Physiol., Lond.* **160**, 106–154 (1962).
2. Bishop, P. O., Coombs, J. S. & Henry, G. H. *J. Physiol., Lond.* **219**, 659–687 (1971).
3. Blakemore, C. & Tobin, E. A. *Expl Brain Res.* **15**, 439–440 (1972).
4. Benevento, L. A., Creutzfeldt, O. D. & Kuhnt, V. *Nature* **238**, 124–126 (1972).
5. Sillito, A. M. *J. Physiol., Lond.* **250**, 305–329 (1975).
6. Tsumoto, T., Eckart, W. & Creutzfeldt, O. D. *Expl Brain Res.* **34**, 351–363 (1979).
7. Ferster, D. *J. Neurosci.* **6**, 1284–1301 (1986).
8. Matsubara, J., Cynader, M., Swindale, N. V. & Stryker, M. P. *Proc. natn. Acad. Sci. U.S.A.* **82**, 935–939 (1985).
9. Ts'o, D. Y., Gilbert, C. D. & Wiesel, T. N. *J. Neurosci.* **6**, 1160–1170 (1986).
10. Kisvarday, Z. F. *et al. Expl Brain Res.* **64**, 541–552 (1986).
11. LeVay, S. *J. comp. Neurol.* **269**, 265–274 (1988).
12. Rose, D. *Vision Res.* **19**, 533–544 (1979).
13. Koch, C. & Poggio, T. in *Models of the Visual Cortex* (eds Rose, D. & Dobson, V. G.) 408–419 (Wiley, New York, 1985).
14. Koch, C. *Soc. Neurosci. Abstr.* **13**, 1451 (1987).
15. Tsumoto, T., Masui, H. & Sato, H. *J. Neurophysiol.* **55**, 469–483 (1986).
16. Hubel, D. H. & Wiesel, T. N. *J. comp. Neurol.* **158**, 267–294 (1974).
17. Albus, K. *Expl Brain Res.* **24**, 181–202 (1975).
18. Schoppmann, A. & Stryker, M. P. *Nature* **293**, 574–576 (1981).
19. Moore, G. P., Segundo, J. P., Perkel, D. H. & Levitan, H. *Biophys. J.* **10**, 876–900 (1970).
20. Bryant Jr, H. L., Marcos, A. R. & Segundo, J. P. *J. Neurophysiol.* **36**, 205–225 (1973).
21. Toyama, K., Kimura, M. & Tanaka, K. *J. Neurophysiol.* **46**, 202–214 (1981).
22. Michalski, A., Gerstein, G. L., Czarkowska, J. & Tarnecki, R. *Expl Brain Res.* **51**, 97–107 (1983).
23. Aertsen, A. H. J. & Gerstein, G. L. J. *Brain Res.* **340**, 341–354 (1985).
24. Matsubara, J. A., Cynader, M. S. & Swindale, N. V. *J. Neurosci.* **7**, 1428–1446 (1987).
25. Somogyi, P., Kisvarday, Z. F., Martin, K. A. C. & Whitteridge, D. *Neuroscience* **10**, 261–294 (1983).
26. Kisvarday, Z. F., Martin, K. A. C., Whitteridge, D. & Somogyi, P. *J. comp. Neurol.* **241**, 111–137 (1985).
27. Blakemore, C., Carpenter, R. H. S. & Georgeson, M. A. *Nature* **228**, 37–39 (1970).
28. Morrone, M. C., Burr, D. C. & Maffei, L. *Proc. R. Soc.* **B216**, 335–354 (1982).
29. Ramoa, A. S., Shadlen, M., Skottun, B. C. & Freeman, R. D. *Nature* **321**, 237–239 (1986).
30. Douglas, R. J., Martin, K. A. C. & Whitteridge, D. *Nature* **332**, 642–644 (1988).

Neuronal correlate of visual associative long-term memory in the primate temporal cortex

Yasushi Miyashita

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

In human long-term memory, ideas and concepts become associated in the learning process[1]. No neuronal correlate for this cognitive function has so far been described, except that memory traces are thought to be localized in the cerebral cortex; the temporal lobe has been assigned as the site for visual experience because electric stimulation of this area results in imagery recall[2] and lesions

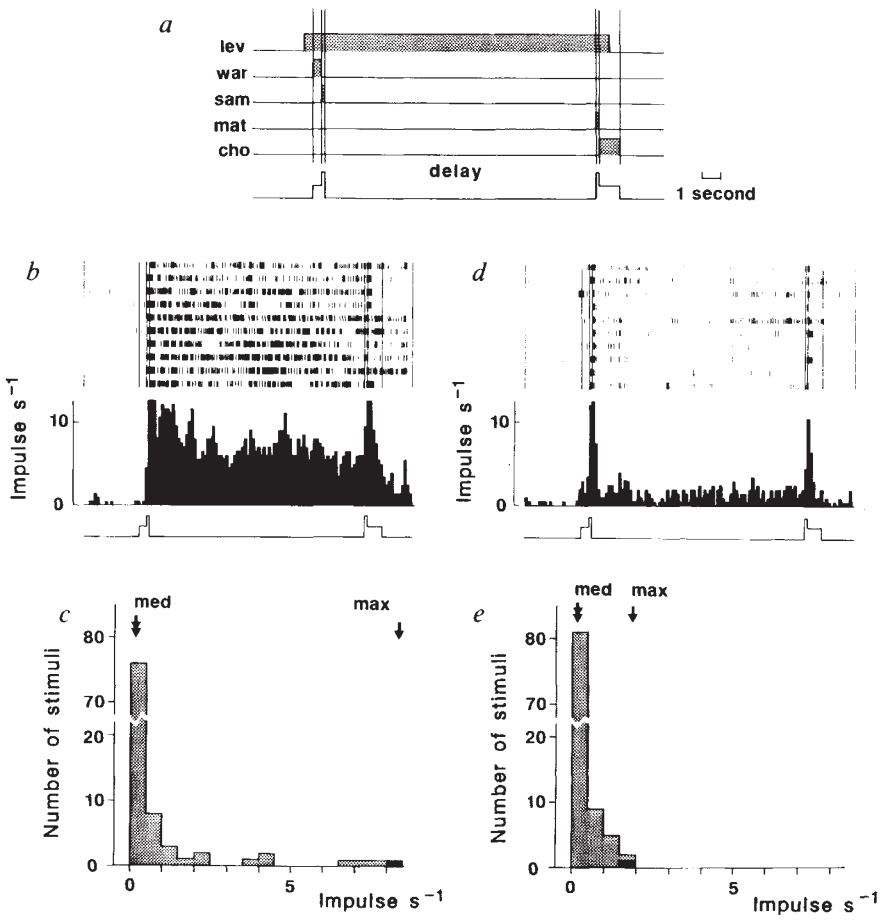

Fig. 1 Neuronal activity in the anterior ventral temporal cortex to the 'learned' and 'new' stimuli in a visual memory task. *a*, Sequence of events in a trial. Lev, lever press by the monkey; war, warning green image; sam, sample stimulus; mat, match stimulus following a 16-s delay; cho, choice signal of white image. Bottom trace, events chart used in *b* and *d*. *b*, Raster recordings of impulse discharges and spike-density histogram obtained from a cell in trials where a 'learned' picture was used as a sample stimulus. These trials were originally separated by intervening trials of other 'learned' or 'new' sample stimuli, and were sorted and collected by off-line computation. Bin width, 200 ms. *c*, Frequency distribution of average firing rate measured during the delay period following 97 'learned' sample pictures in the same cell. The black column indicates the response shown in *b*. ↓ max and ↓med, maximum and median value in the distribution. The selectivity of this neuron to these learned pictures, measured by the kurtosis of the response distribution, was 11.1. The kurtosis (ref. 13) is defined as: $N^{-1}(\sum_{i=1}^{N}((x_i-\bar{x})/s)^4)-3$, where x_i is the response to the ith picture, $\bar{x}$ and s are the mean and standard deviation of the distribution, N is the total number of pictures tested. *d*,*e*, Similar to *b*,*c* but 'new' pictures are used as sample stimuli in the same cell. The black column in *e* represents the response illustrated in Fig. 1*d*. The kurtosis of the response distribution shown in *e* was 5.2.

Methods. In our modified delayed matching-to-sample (DMS) task[10], sample and match stimuli were successively presented on a video monitor, each for 0.2 s at a 16-s delay interval. The trials with identical or non-identical match stimulus were mixed randomly. Drops of fruit juice were administered as a reward. After the monkey had learned the DMS rule to an 85% performance level, he was over trained for two weeks with a fixed set of 97 fractal patterns ('learned stimuli') presented in a fixed sequence according to an arbitrarily attached number (serial position number, SPN). The same training was carried out throughout the search period. During the unit recording, a new set of patterns ('new stimuli') was created for each neuron and also formally given SPNs. The sample and match stimuli were then selected at random from the 'learned' and 'new' patterns, independent of the SPNs. The monkeys' performance level did not differ significantly for 'learned' and 'new' stimuli (85–100% correct). Error trials were excluded from the analysis in this report.

produce deficits in visual recognition of objects[3–9]. We previously reported that in the anterior ventral temporal cortex of monkeys, individual neurons have a sustained activity that is highly selective for a few of the 100 coloured fractal patterns used in a visual working-memory task[10]. Here I report the development of this selectivity through repeated trials involving the working memory. The few patterns for which a neuron was conjointly selective were frequently related to each other through stimulus–stimulus association imposed during training. The results indicate that the selectivity acquired by these cells represents a neuronal correlate of the associative long-term memory of pictures.

Two adult monkeys (*Macaca fuscata*) were trained to perform a visual memory task[10] (Fig. 1*a*). Each monkey memorized a sample stimulus during a delay period and then had to decide whether a second stimulus was the same as the sample. A set of 97 colour patterns was generated by a fractal algorithm with a 32-bit seed of random numbers[11]; the set was repeatedly used during an over-training session ('learned stimuli'). While extracellular neural discharges were recorded using standard physiological techniques[12], a sample stimulus was selected not only from the 97 learned patterns but also from a new set of 97 patterns ('new stimuli'). Different sets of new stimuli were created for each neuron using the same algorithm but a different seed. The 'learned' and 'new' stimuli were used at random.

A few of the 97 learned stimuli reproducibly activated a particular neuron with high-frequency sustained discharges during the delay period of the task[10] (Fig. 1*b* and *c*). The optimal set of stimuli varied from cell to cell. By contrast, the 97 new patterns produced only weak delay responses (Fig. 1*d* and *e*). No systematic tendency was found for the delay discharges to a new sample stimulus to become augmented or suppressed with up to 10 repetitions (Fig. 1*d*). The distribution of the delay discharge rate to the new stimuli (Fig. 1*e*) lacked the small population of high-frequency responses (>2 impulse s^{-1}) that characterized the distribution of responses to the learned stimuli (Fig. 1*c*).

Of the 206 neurons recorded in the anterior ventral temporal cortex of the over-trained monkeys, 57 neurons exhibited shape-selective delay discharges as described previously[10], and 17 of the 57 cells were successfully tested with both learned and new stimuli. In these 17 cells, the maximum delay discharge for learned stimuli (for example the arrow in Fig. 1*c*) was always larger than that for new stimuli (for example the arrow in Fig. 1*e*), and the difference was highly significant ($P<0.001$, Wilcoxon test). When the selectivity of a neuron to a set of 97 patterns was represented by the sharpness of the response distribution (the kurtosis of the distribution[13], see Fig. 1*c* legend), the selectivity to the learned patterns was almost always (15/17) higher than that to the new patterns ($P<0.005$). Despite the presence of a few effective pictures in the learned stimuli, there was no difference between the median responses to the learned and new stimuli (for example the double arrows in Fig. 1*c* and *e*) for the 17 cells ($P>0.5$).

The effectiveness of the learned stimuli cannot be due to any special feature of their geometric patterns for two reasons: (1) the set of learned stimuli and each set of new stimuli were artificially generated by the same fractal algorithm[11], thus they belonged to the same class of geometric patterns; (2) different sets of learned stimuli assigned to each monkey yielded identical results. Therefore the sharpness of the response selectivity of these neurons to the learned patterns was most likely to have been formed throughout training.

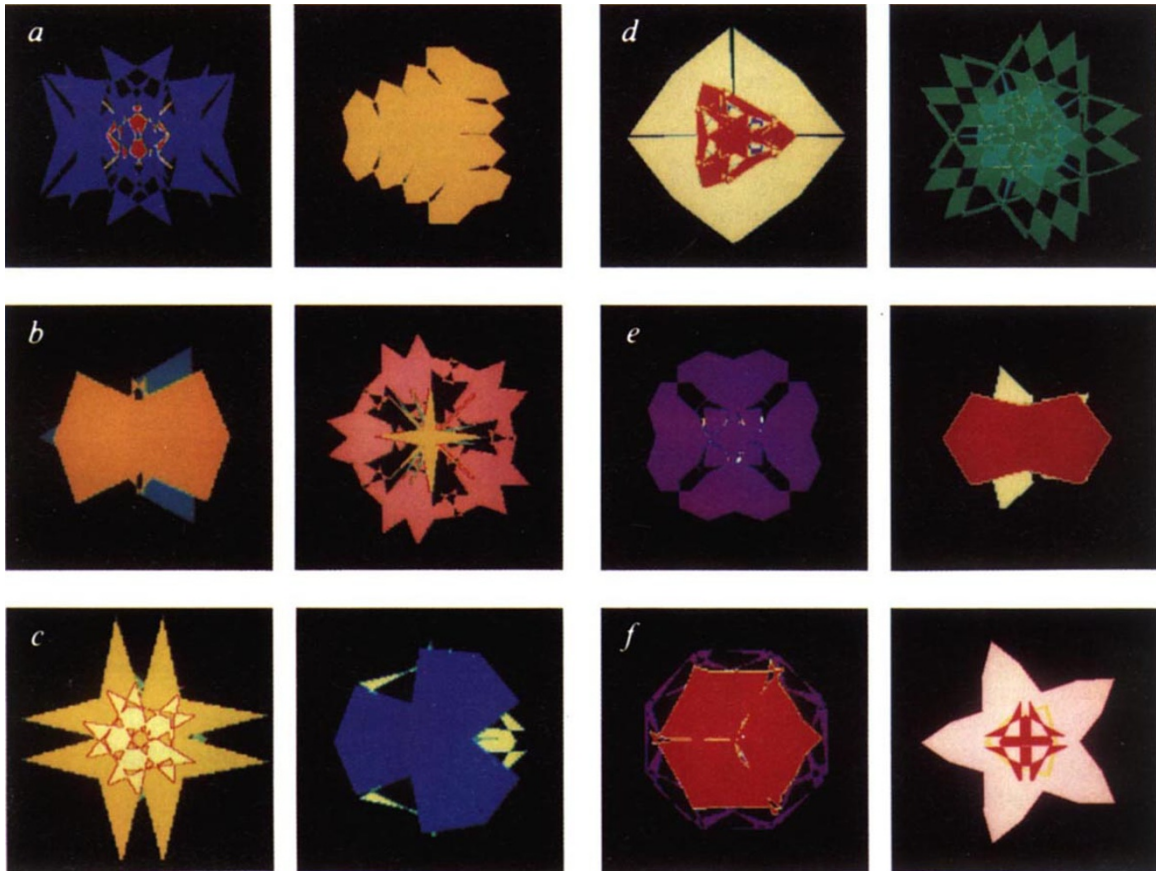

Fig. 2 Coloured fractal patterns that produced the two strongest delay discharges in learned stimuli for six different cells (*a*–*f*). The two optimal stimuli shown for each neuron have no similarities in their geometric patterns.

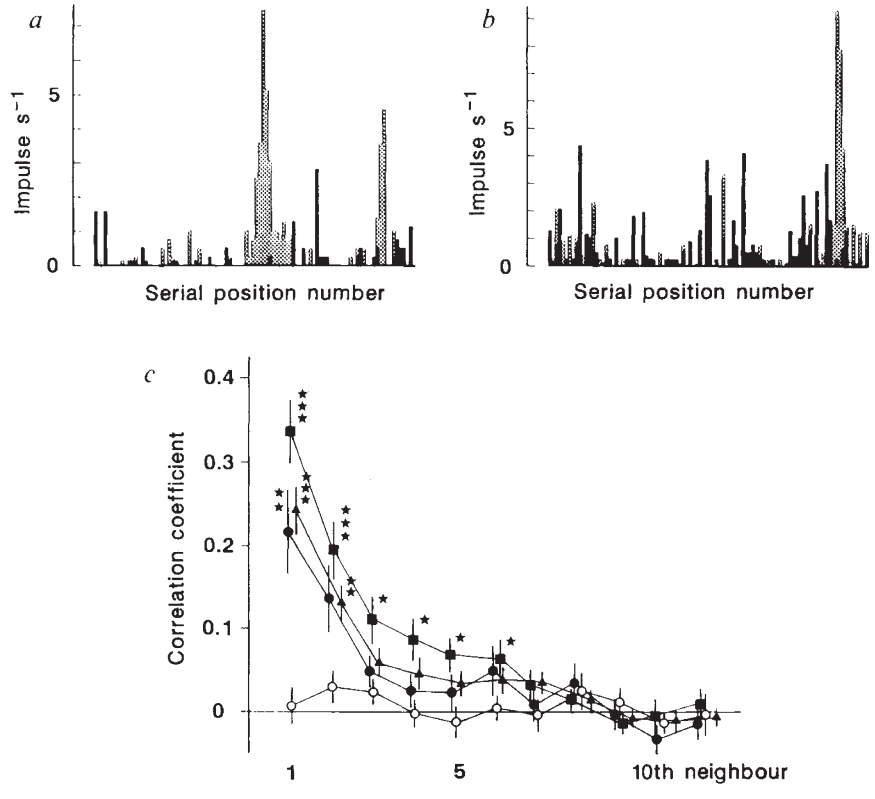

Fig. 3 Stimulus–stimulus association among the learned fractal patterns. *a*, Average delay discharge rate (impulse s^{-1}) for each sample stimulus in a cell against serial position number (SPN) of the stimuli (see Fig. 1 legend). Stippled columns, learned stimuli; black columns, new stimuli. *b*, As *a*, but for a different cell. *c*, Autocorrelograms of the delay discharge rate along the SPN of the stimuli. ● and ○, Average autocorrelogram for the learned and new stimuli in the 17 cells; ▲, that for the learned stimuli in the 57 cells; ■, that for the learned stimuli in the 28 cells for which the nearest-neighbour correlation along the SPN was significant ($P<0.05$) according to Kendall's test. Error bars, standard errors. Three vertical asterisks $P<0.001$, two vertical asterisks, $P<0.01$, one asterisk, $P<0.05$, according to the Kolmogorov–Smirnov test in comparison with the value for new stimuli.

Is it possible that training determines not only how sharply the effective learned patterns are represented in each neuron (as shown above), but also which patterns are conjointly chosen as the few optimal stimuli. I examined the geometric similarities between the optimal stimuli of a cell, and found that the stimuli were often completely different (Fig. 2), indicating that there is a non-geometric criterion for choice of these patterns as the optimal stimuli of a cell. I then examined the effect of a fixed-order presentation of the patterns during the training session according to an arbitrarily attached serial position number (SPN; see Fig. 1 legend). I expected that if the consecutively presented patterns tended to be associated together and if the association was fixed in the choice of effective patterns for a cell, the effective patterns would be correlated along the SPN, in spite of a random presentation of the stimuli during the unit-recording session.

Figure 3 shows that the effective responses to the learned stimuli do indeed cluster along the SPNs (*a* and *b*, stippled columns). The clustering was not due to an artefact in the testing procedure because the responses simultaneously obtained from the new stimuli were not clustered (black columns). In the 17 cells that were tested with both learned and new stimuli, the responses to the learned stimuli were significantly correlated (Fig. 3*c*, ●) in the nearest-neighbour of the SPNs, compared with the responses to the new stimuli (○) ($P<0.01$, Kolmogorov–Smirnov test). The 57 cells tested by the learned stimuli had similar correlated responses along the SPN (▲). The nearest-neighbour correlations for the learned stimuli differed from cell to cell, and were significant ($P<0.05$) in 28 of the 57 cells according to Kendall's correlation test[13], whereas those for the new stimuli were not significant in any of the 17 cells. The autocorrelogram along the SPNs in these 28 cells (■) differed significantly up to the sixth neighbour from that of the new stimuli, but not from the seventh neighbour onwards ($P>0.2$). This implies that the cells could associate pictorial patterns for the six consecutive neighbours during learning.

In the primate inferior temporal (IT) cortex, neural discharges selective to specific complex objects have been reported for hands[6,14,15], faces[15,16], Fourier descriptors[17] and geometric patterns used in a discrimination task[18]. However, the selectivity of these sensory responses seemed to be organized along the patterns' geometric similarity[14–18]. The neurons reported here were selective for the artificial fractal patterns and had two distinct properties compared with other shape-selective IT neurons. First, these neurons were active in the delay period of the working-memory task, that is, when the visual stimulus was off and the monkey memorized it. Second, most other sensory neurons were located posterior to the area explored here, TE_{av} (ref. 19) (or TE_1–TE_2 (ref. 20)). TE_{av} has been anatomically designated as the last link from the visual system to the limbic memory systems[7,19,21,22], especially the hippocampus[23]. It is tempting to assume that this interaction with the limbic system is important in organizing neuronal selectivity for the association of geometrically dissimilar pictures.

The work presented here shows directly that the responses of a single IT neuron reflect an experimentally controlled association between a temporally related set of stimuli. There are indirect clues indicating that there is such association in daily life; in the polysensory areas deep within the superior temporal sulcus (mainly area TPO)[20], which receive a projection from the IT cortex[24], some cells were selective for a person's different perspective views (face or body movement)[25], which might be interpreted as an associative experience between the perspective views that are visually distinct but often temporally related. Some face neurons did indeed change their responses when new faces were repeatedly shown[26]. Visual associative agnosia[3,27,28] in humans may result from selective loss of the neurons, thereby encoding the association between visually dissimilar objects.

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1. Anderson, J. R. & Bower, G. H. *Human Associative Memory* (Lawrence Erlbaum Associates Hillsdale, 1980).
2. Penfield, W. & Perot, P. *Brain* **86,** 595–697 (1963).
3. McCarthy, R. A. & Warrington, E. K. *J. Neurol. Neurosurg. Pschiat.* **49,** 1233–1240 (1986).
4. Kimura, D. *Arch. Neurol.* **8,** 48–55 (1963).
5. Milner, B. *Neuropsychologia* **6,** 191–209 (1968).
6. Gross, C. G. in *Handbook of Sensory Physiology* Vol. 8, part 3B (ed. Jung, R.) 451–482 (Springer, Berlin, 1972).
7. Mishkin, M. *Phil. Trans. R. Soc.* **B298,** 85–95 (1982).
8. Gaffan, D. & Weiskrantz, L. *Brain Res.* **196,** 373–386 (1980).
9. Sahgal, A., Hutchison, R., Hughes, R. P. & Iverson, S. D. *Behavl Brain Res.* **8,** 361–373 (1983).
10. Miyashita, Y. & Chang, H. S. *Nature* **331,** 68–70 (1988).
11. Miyashita, Y. in *Competition and Cooperation of Neural Nets* Vol. 2 (ed. Arbib, M.) (Springer, Berlin, in the press).
12. Miyashita, Y. & Nagao, S. *J. Physiol.* **351,** 251–262 (1984).
13. Snedecor, G. W. & Corcoran, W. G. *Statistical Method* (Iowa University Press, Iowa, 1980).
14. Gross, C. G., Rocha-Miranda, C. E. & Bender, D. B. *J. Neurophysiol.* **35,** 96–111 (1972).
15. Desimone, R., Albright, T. D., Gross, C. G. & Bruce, C. *J. Neurosci.* **4,** 2051–2062 (1984).
16. Rolls, E. T. & Baylis, G. C. *Brain Res.* **65,** 38–48 (1986).
17. Schwartz, E. L., Desimone, R., Albright, T. D. & Gross, C. G. *Proc. natn. Acad. Sci. U.S.A.* **80,** 5776–5778 (1983).
18. Sato, T., Kawamura, T. & Iwai, E. *Expl Brain Res.* **38,** 313–319 (1980).
19. Turner, B. H., Mishkin, M. & Knapp, M. *J. comp. Neurol* **191,** 515–543 (1980).
20. Seltzer, B. & Pandya, D. N. *Brain Res.* **149,** 1–24 (1978).
21. Herzog, A. G. & Van Hoesen, G. W. *Brain Res.* **115,** 57–69 (1976).
22. Van Hoesen, G. W. & Pandya, D. N. *Brain Res.* **95,** 1–24 (1975).
23. Yukie, M. *Neurosci. Res.* Suppl. **7,** S16 (1988).
24. Jones, E. G. & Powell, T. P. S. *Brain* **93,** 793–820 (1970).
25. Perret, D. I., Mistlin, A. J. & Chitty, A. J. *Trends Neurosci.* **10,** 358–364 (1987).
26. Baylis, G. C., Hasselmo, M. E. & Rolls, E. T. *Soc. Neurosci.* Abstr. **13,** 1317 (1987).
27. Hecaen, H., Goldblum, M. C., Masure, M. C. & Ramier, A. M. *Neuropsychologia* **12,** 447–464 (1974).
28. Marshall, J. C. *Nature* **334,** 378 (1988).

Persistent protein kinase activity underlying long-term potentiation

Roberto Malinow*, Daniel V. Madison* & Richard W. Tsien*

Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut 06510, USA

Long-term potentiation (LTP) of synaptic transmission in the hippocampus is a much-studied example of synaptic plasticity[1,2]. Although the role of N-methyl-D-aspartate (NMDA) receptors in the induction of LTP is well established[3–5], the nature of the persistent signal underlying this synaptic enhancement is unclear. Involvement of protein phosphorylation in LTP has been widely proposed[6–15], with protein kinase C (PKC)[6–8,10–12,14] and calcium-calmodulin kinase type II (CaMKII)[9,13] as leading candidates. Here we test whether the persistent signal in LTP is an enduring phosphoester bond, a long-lived kinase activator, or a constitutively active protein kinase by using H-7, which inhibits activated protein kinases[16] and sphingosine, which competes with activators of PKC (ref. 17) and CaMKII (ref. 18). H-7 suppressed established LTP, indicating that the synaptic potentiation is sustained by persistent protein kinase activity rather than a stably phosphorylated substrate. In contrast, sphingosine did not inhibit established LTP, although it was effective when applied before tetanic stimulation. This suggests that persistent kinase activity is not maintained by a long-lived activator, but is effectively constitutive. Surprisingly, the H-7 block of LTP was reversible; evidently, the kinase directly underlying LTP remains activated even though its catalytic activity is interrupted indicating that such kinase activity does not sustain itself simply through continual autophosphorylation (see refs 9, 13, 15).

We studied LTP in the Schaffer collateral/commissural-CA1 pathway in rat hippocampal slices. Following a conditioning tetanus, post-tetanic potentiation decayed within ~5 min and was followed by a more slowly decaying potentiation that disappeared within about 30 min (Fig. 1*a*; refs 4, 19). These com-

* Present address: Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, California 94305, USA.