5. Andersson, M. in *Producers and Scroungers: Strategies of Exploitation and Parasitism* (ed. Barnard, C. J.) 195-228 (Croom Helm, London, 1984).
6. Yom-Tov, Y. *Biol. Rev.* **55**, 93-108 (1980).
7. Brown, C. R. *Science* **234**, 83-85 (1986).
8. American Ornithologist's Union, *Check-list of North American Birds, 6th edn* (Lawrence, Kansas, 1983).
9. Feare, C. *The Starling* (Oxford University Press, Oxford, 1984).
10. Friedmann, H. *The Cowbirds* (Thomas Springfield, Illinois, 1929).
11. Hamilton, W. J. III & Orians, G. H. *Condor* **67**, 361-382 (1965)
12. Brown, C. R. thesis, Univ. Princeton, (1985).
13. Gillespie, J. H. *Genetics* **76**, 601-606 (1974).
14. Payne, R. B. *A. Rev. Ecol. Syst.* **8**, 1-28 (1977).
15. Blomme, C. *Ontario Field Biol.* **37**, 34-35 (1983).
16. Truslow, F. K. *Living Bird* **6**, 227-236 (1967).
17. Trost, C. H. & Webb, C. L. *Anim. Behav.* **34**, 294-295 (1986).

Neuronal correlate of pictorial short-term memory in the primate temporal cortex

Yasushi Miyashita & Han Soo Chang

Department of Physiology, Faculty of Medicine, University of Tokyo, Hongo, Tokyo 113, Japan

It has been proposed that visual-memory traces are located in the temporal lobes of the cerebral cortex, as electric stimulation of this area in humans results in recall of imagery[1]. Lesions in this area also affect recognition of an object after a delay in both humans[2,3] and monkeys[4–7], indicating a role in short-term memory of images[8]. Single-unit recordings from the temporal cortex have shown that some neurons continue to fire when one of two or four colours are to be remembered temporarily[9]. But neuronal responses selective to specific complex objects[10–18], including hands[10,13] and faces[13,16,17], cease soon after the offset of stimulus presentation[10–18]. These results led to the question of whether any of these neurons could serve the memory of complex objects. We report here a group of shape-selective neurons in an anterior ventral part of the temporal cortex of monkeys that exhibited sustained activity during the delay period of a visual short-term memory task. The activity was highly selective for the pictorial information to be memorized and was independent of the physical attributes such as size, orientation, colour or position of the object. These observations show that the delay activity represents the short-term memory of the categorized percept of a picture.

In a trial of our visual short-term memory task, sample and match stimuli were successively presented on a video monitor, each for 0.2 s at a 16 s delay interval (Fig. 2*a*). The stimuli were newly selected for each trial among 100 computer-generated coloured fractal patterns (examples are shown in Fig. 1) and 100 pseudo-coloured images of scenery. Two monkeys (*Macaca fuscata*) were trained to memorize the sample stimulus and to decide whether the match stimulus was the same or different (see legend to Fig. 2). Extracellular spike discharges of 188 neurons were recorded from the anterior ventral part of the temporal cortex (Fig. 2*b*) of these monkeys with standard physiological techniques[19]. Recording of electro-oculographs revealed no systematic differences in eye position which could be related to differential neural responses described below.

Figure 2*c* shows reproducible stimulus-dependent discharges during the delay obtained in one cell for four different sample stimuli (Fig. 1*a–d*) (prominent in *a* and *b*, but virtually ineffective in *c* and *d*). A time course of the delay activity in Fig. 2*ci* is shown in Fig. 2*d*, as contrasted with those for six other ineffective stimuli (Fig. 1*c–h*). These histograms are representative of those accumulated with 57 other sample stimuli. Only two of the 64 tested stimuli (Fig. 2*e*) were followed by especially high delay activity (>10 impulses $\cdot s^{-1}$), shown in Fig. 2*ci* and ii.

These delay activities do not represent mere sensory after-discharge[9,11] for the following reasons. First, the high rate of firing did not decline throughout the whole 16 s delay period (Fig. 2*d*). Second, firing frequency exhibited during the delay was not necessarily correlated with that during the stimulus presentation (Fig. 2*c* and *d*). Third, the delay activity in some neurons started after a latency of a few seconds following stimulus presentation (data not shown). Thus, it is concluded that the delay activity is not a passive continuation of the firing during the sensory stimulation, but represents a mnemonic activity to retain visual information.

Of the 188 neurons tested, 144 showed a correlation between firing and one or more events of the trials. Among the 144 cells, 95 showed a sustained increase or decrease of discharge frequency during the delay period, whereas the others fired only during stimulus presentation. In 77 of these 95 cells, the discharge frequency varied depending on sample stimuli, but the remaining 18 did not exhibit such selectivity. In many of the 77 selective cells, only a few pictures elicited a strong delay activation such as shown in Fig. 2*c* and *d*. It is notable that the optimal picture differed from cell to cell, and that the whole population of the optimal pictures for the 77 cells covered a substantial part of the repertory of 200 pictorial stimuli.

For further analysis of triggering features of the delay responses, sample pictures were manipulated in the following way (for example Fig. 1*i–l*): (1) stimulus size was reduced by half, (2) stimuli were rotated by 90° in a clockwise direction, (3) coloured stimuli were transformed into monochrome by referring to a pseudo-colour look-up table, and (4) stimulus position was changed on the video monitor (data not shown) (a 0.2 s stimulus presentation time is short enough to exclude the contribution of saccadic eye movement). Figure 3 shows responses of a neuron which consistently fired during the delay after one particular picture (shown in Fig. 1*i–l*) but not after others, irrespective of stimulus size (Fig. 3*a*ii and *b*ii), orientation (Fig. 3*a*iii and *b*iii), or colour (Fig. 3*a*iv and *b*iv). Similar tolerance of responses was observed in a majority of the tested delay neurons: to size in 16 out of 19 cells, to orientation in 5 out of 7 cells, to colour-monochrome in 15 out of 20 cells, and to position in 8 out of 13 cells. In other neurons, manipulation of the most effective stimulus reduced or abolished the delay discharge.

In the inferior temporal cortex, shape-selective neuronal discharges have been reported for Fourier descriptors[12], face[13,16,17], hands[10,13] or stimuli used in a discrimination task[14,15], although all of these were sensory responses evoked during presentation of stimulus. The relative selectivity of these sensory neurons remained invariant over changes of size, position, orientation or contrast[12–15,17]. The present delay responses could be derived through such sensory responses, inheriting from them the tolerance to such stimulus transformations. It is notable that, for the Fourier descriptor neurons[12], the absolute level of the response varied widely over changes in stimulus size, although this was not the case for many of the present neurons. This may suggest that the present neurons represent more abstract properties of objects (like shape percept) than do such sensory neurons.

In the colour-selective delay neurons previously described[9], time courses of sustained delay discharge were similar to those found in the present shape-selective delay neurons (compare Fig. 2*d* or Fig. 3*a* with Fig. 7 or Fig. 6 of ref. 9). The differential delay activity in the colour task was mainly found in the cortex of the lower bank of the superior temporal sulcus[9], lying more posteriorly and dorsally to the presently explored area, and the colour cells seemed to be scattered[9], whereas the present cells tended to group in smaller areas.

A majority of our shape-selective delay neurons were recorded in TE_{av}[20] (or TE_1-TE_2[21]) and some in TG_v[20] and in area 35. These areas are anatomically designated as the last link from the visual system to limbic memory systems[4,20,22,23]. Neurons in these areas were visually responsive with a large receptive field[24]. Impairment of the recognition memory task resulted from lesions including these areas[4–7], consistent with the presently-postulated mnemonic role of neurons in these areas.

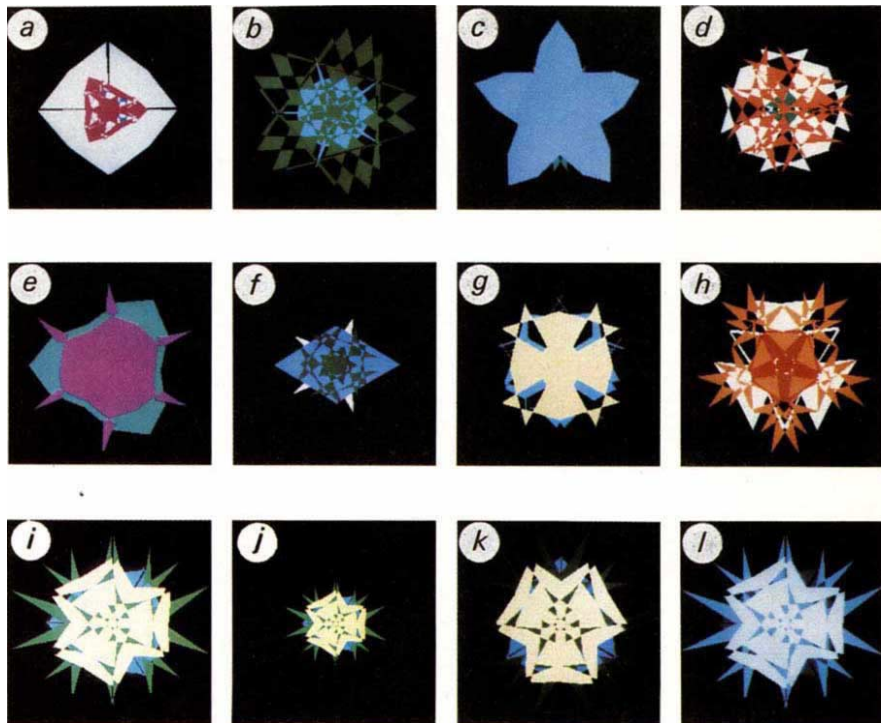

Fig. 1 Examples of coloured fractal patterns. *a–h*, Stimuli used in the trials of Figs 2*c* and *d*. Panels *i–l*, illustrate size reduction (*j*), rotation (*k*) and colour-monochrome transformation (*l*) of stimulus (*i*), as used in the test shown in Fig. 3.

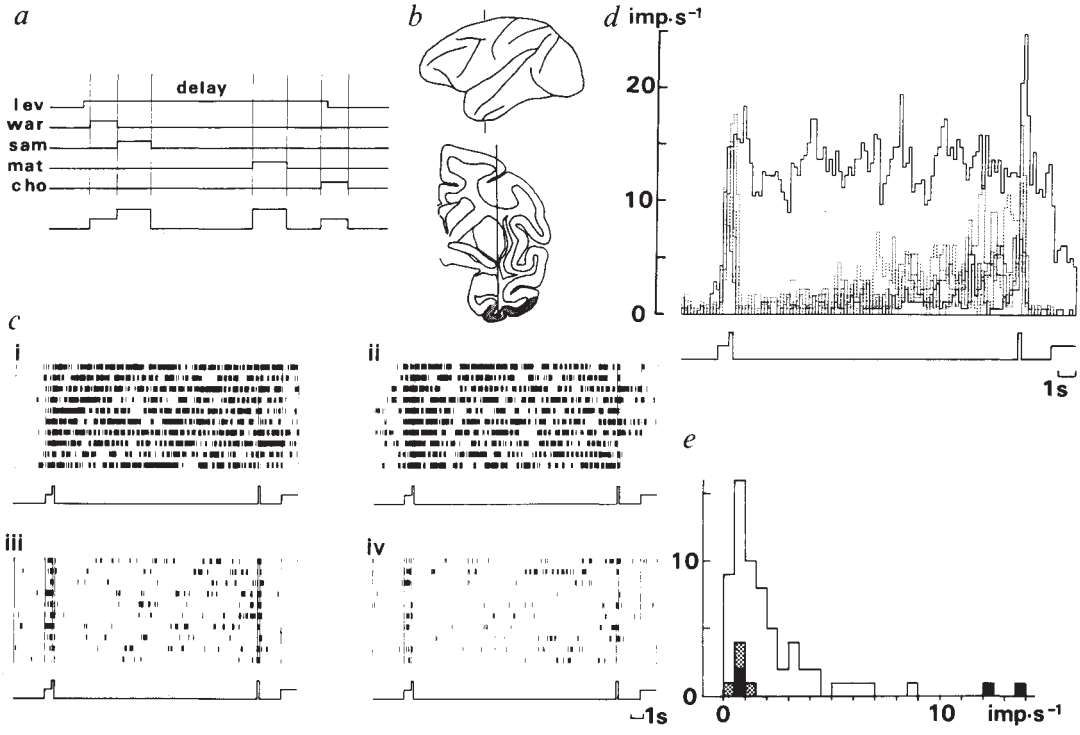

Fig. 2 Responses of a neuron in the anterior ventral temporal cortex in a visual short-term memory task. *a*, Sequence of events in a trial (Lev, lever press by the monkey; War, warning green image (0.5 s); Sam, sample stimulus (0.2 s); Mat, match stimulus (0.2 s) following a delay of 16 s; Cho, choice signal of white image). Lowest trace, the events-chart used in Fig. 2*c–d* and Fig. 3*a*. *b*, Location of recording sites. Top, a lateral view of a macaque brain. Bottom, a section indicated by a vertical line on the lateral view. The stippled area represents the range of recording sites. The vertical line and the dot at its end represent the microelectrode track and the position of the cell shown in Fig. 2*c–e*. *c*, Raster recordings of impulse discharge from a cell. i, Trials whose sample stimulus was as shown in Fig. 1*a*. ii, iii and iv, Obtained as in Fig. 1*b*, *c* and *d*, respectively. Trials of the same sample stimulus were originally separated by intervening trials of other sample stimuli, and these were sorted and collected by off-line computation. *d*, Spike-density histograms for the delay activity of Fig. 2*ci* and for those for six other ineffective stimuli (Fig. 1*c–h*). Seven to 16 trials were accumulated for each sample stimulus. Bin width, 200 ms. *e*, Distribution of average delay spike frequencies following 64 different sample pictures, with which more than four trials were tested. The same cell as in *c* and *d*. Ordinate, number of sample pictures used as stimuli. Closed columns, responses shown in Fig. 2*c*. The stippled columns, other responses included in Fig. 2*d*.

Method. Each sample picture was paired with a match stimulus that was identical and one that was not identical. For each trial, the identical or non-identical match stimulus was assigned randomly. If the match stimulus was different from the sample, the monkey had to release the lever and touch the video screen to obtain fruit juice. If the match stimulus was the same as the sample, the monkey had to keep pressing the lever until the choice signal was turned off. If the monkey released the lever before the choice signal, the trial was cancelled. The monkeys' decisions were 85–100% correct. Error trials were excluded from the analysis presented here. In training sessions and the search period stimuli were presented in a fixed sequence. In the recording session, a long sequence of trials using the entire repertory of stimuli was run repeatedly. When some pictures were found to elicit stronger responses than others, the relevant pictures were selected and shorter sequences run with fewer stimuli.

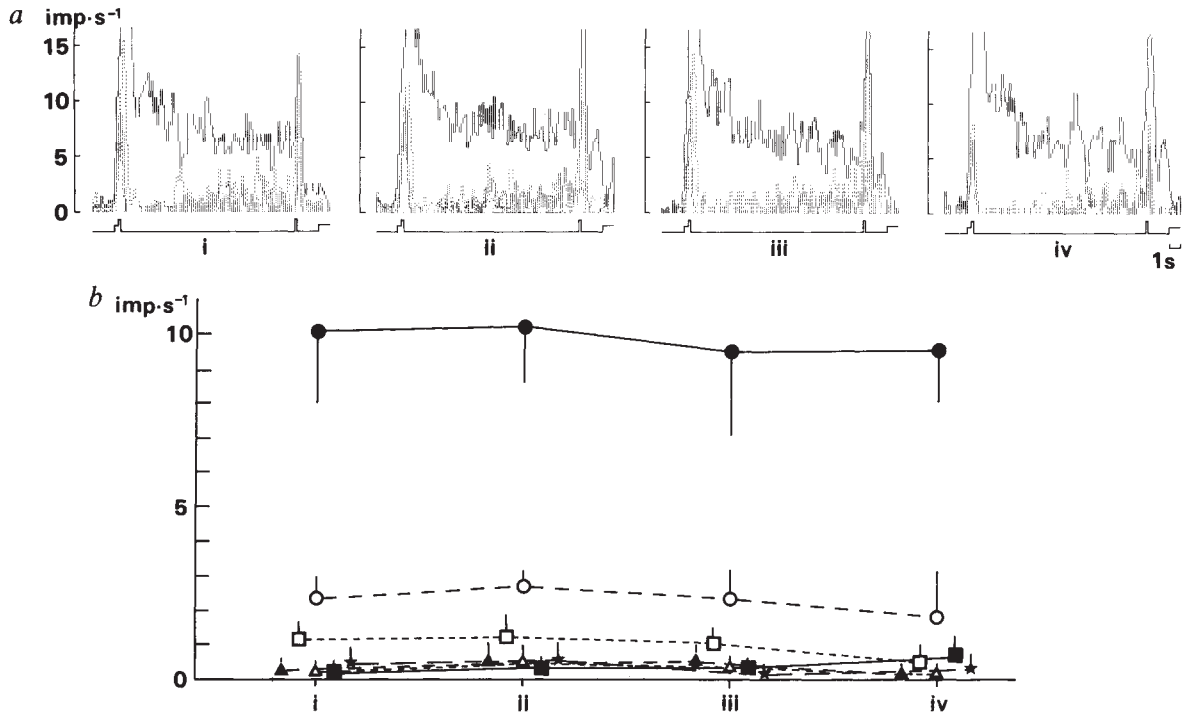

Fig. 3 Response invariance under stimulus transformation in size, orientation or colour. A different cell from that in Fig. 2. *a*, Histograms similar to that in Fig. 2*d*, but for five different sample pictures. i, Control responses. Note that the sample stimulus of Fig. 1*i* elicited the strongest delay activity in this cell. ii, iii and iv, Effects of stimulus size reduction by half (for example Fig. 1*j*), of stimulus rotation by 90° in a clockwise direction (for example Fig. 1*k*) and of colour-to-monochrome transformation (for example Fig. 1*l*). *b*, Average delay spike frequencies as a function of stimulus transformation (i, original; ii, size reduction; iii, rotation; iv, colour to monochrome). Responses to seven different sample pictures (including five shown in *a*) are plotted with different symbols. ●, Responses to stimuli of Fig. 1*i–l*. Error bars indicate standard deviations for 4–15 trials.

The present results suggest that pictorial short-term memory is coded by temporary activation of an ensemble of neurons in the region of the association cortex that processes visual information[4,9,25], rather than by neuronal activity in a brain area specialized for short-term memory. Although each neuron in the ensemble has highly abstract and selective coding features, representation of the memory of a picture seems to be distributed among a number of neurons. We need to know how the distributed information is decoded for subsequent decision processes[26].

We thank Professor Masao Ito for his encouragement.

Received 3 July; accepted 5 November 1987.

1. Penfield, W. & Perot, P. *Brain* **86**, 595–697 (1963).
2. Kimura, D. *Arch. Neurol.* **8**, 48–55 (1963).
3. Milner, B. *Neuropsychologia* **6**, 191–209 (1968).
4. Mishkin, M. *Phil. Trans. R. Soc.* B **298**, 85–95 (1982).
5. Gaffan, D. & Weiskrantz, L. *Brain Res.* **196**, 373–386 (1980).
6. Sahgal, A., Hutchinson, R., Hughes, R. P. & Iverson, S. D. *Behav. Brain Res.* **8**, 361–373 (1983).
7. Fuster, J. M., Bauer, R. H. & Jervey, J. P. *Expl Neurol.* **71**, 398–409 (1981).
8. Warrington, E. K. & Shallice, T. *Quart. J. exp. Psychol.* **24**, 30–40 (1972).
9. Fuster, J. M. & Jervey, J. P. *J. Neurosci.* **3**, 361–375 (1982).
10. Gross, C. G., Rocha-Miranda, C. E. & Bender, D. B. *J. Neurophysiol.* **35**, 96–111 (1972).
11. Gross, C. G., Bender, D. B. & Mishkin, M. *Brain Res.* **131**, 227–239 (1977).
12. Schwartz, E. L., Desimone, R., Albright, T. D. & Gross, C. G. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5776–5778 (1983).
13. Desimone, R., Albright, T. D., Gross, C. G. & Bruce, C. *J. Neurosci.* **4**, 2051–2062 (1984).
14. Sato, T., Kawamura, T. & Iwai, E. *Expl Brain Res.* **38**, 313–319 (1980).
15. Iwai, E. *Vision Res.* **25**, 425–439 (1985).
16. Perret, D. I., Rolls, E. T. & Caan, W. *Expl Brain Res.* **47**, 329–342 (1982).
17. Rolls, E. T. & Baylis, G. C. *Brain Res.* **65**, 38–48 (1986).
18. Baylis, G. C. & Rolls, E. T. *Expl Brain Res.* **65**, 614–622 (1987).
19. Miyashita, Y. & Nagao, S. *J. Physiol., Lond.* **351**, 251–262 (1984).
20. Turner, B. H., Mishkin, M. & Knapp, M. *J. comp. Neurol.* **191**, 515–543 (1980).
21. Seltzer, B. & Pandya, D. N. *Brain Res.* **149**, 1–24 (1978).
22. Herzog, A. G. & Van Hoesen, G. W. *Brain Res.* **115**, 57–69 (1976).
23. Van Hoesen, G. W. & Pandya, D. N. *Brain Res.* **95**, 1–24 (1975).
24. Desimone, R. & Gross, C. G. *Brain Res.* **178**, 363–380 (1979).
25. Anderson, J. R. *Cognitive Psychology and its Implications* (Freeman, San Francisco, 1980).
26. Coltheart, M. *Phil. Trans. R. Soc.* B **302**, 283–294 (1983).

A complete culture system for the chick embryo

M. M. Perry

Gene Expression Group, AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS, UK

The embryonic lifespan of the chick is 22 days. Development in the first day takes place in the oviduct, and in the remaining 21 days in the shelled egg. There have been few attempts to culture oviductal embryos[1–3], though methods covering the first few days of development *in ovo* are well established[4] and a method for the final 18 days of development through hatching has recently been devised[5]. I have now succeeded in culturing the fertilized ovum of the chick (*Gallus domesticus*) for the total embryonic period by growing it in a series of separate culture systems. This is the first report of a complete *in vitro* method for a homoiothermic animal. The technique opens the way to the investigation of developmental events in birds that require access to the embyro at the single-cell stage, and in particular to the genetic manipulation of the fertilized ovum.

Chick development has been divided into three periods for present purposes: fertilization to blastoderm formation (I) lasts for one day[6], embryogenesis (II) lasts for three days and embryonic growth (III) for eighteen days[7]. Fertilization takes place in the anterior oviduct, after which the yolk-laden ovum is encased in albumen secreted by the magnum. Around the time of the first division of the zygote, some 4.5 h after ovulation, the shell membrane is deposited in the isthmus and the albumen