

a bladder carcinoma that grows rapidly in syngeneic Wistar rats. Immunization with BERH-2-B cells did not inhibit the growth of NBT-II cells *in vivo* (Table 2). In addition, CD8<sup>+</sup> T cells from rats immunized with BERH-2-B lysed BERH-2 cells but not NBT-II cells *in vitro* (12).

Finally, we determined whether *in vitro* selection of hybrid tumor cells was obligatory for the induction of tumor immunity. After fusing BERH-2 tumor cells with activated B cells, we washed the mixture of cells and injected them subcutaneously into syngeneic rats without prior *in vitro* selection. The efficiency of the fusion ranged from 30 to 50%. For controls, we injected BERH-2 tumor cells mixed with activated B cells in the absence of PEG. All animals were then injected with the parental BERH-2 cells intrahaptically. Only animals immunized with tumor cells fused with activated B cells were protected from tumor formation. Simply mixing tumor cells with activated B cells was not effective in inducing protective immunity (Fig. 2D), nor was treating BERH-2 tumor cells with PEG alone (12).

In summary, a BERH hepatocarcinoma-specific vaccine in rats can be made by fusing tumor cells with syngeneic, activated B cells. In addition to MHC class II and B7 antigens, BERH-2-B cells may express other cell surface molecules that are essential for the stimulation of host T cells. Production of B cell-specific cytokines by hybrid tumor cells may be important in the elicitation of host immune responses (13). BERH-2 cells fused with activated T cells were unable to stimulate BERH-2-specific immune responses (12). Preliminary experiments suggest that tumor cells fused with activated allogeneic B cells are also immunogenic and can induce protective immunity (12).

In order to induce protective immunity, the hybrid tumor cells must retain their capacity to express tumor-specific antigens. In addition, the hybrid tumor cells must be able to process and present tumor-specific antigens so as to activate host T cells. Whether this approach can be used in other tumor models remains to be determined. Our observation that protective immunity can be induced by tumor cells fused with activated B cells without *in vitro* selection may have broad clinical applications and may provide a useful strategy for cancer immunotherapy.

## REFERENCES AND NOTES

- M. E. Osband and S. Ross, *Immunol. Today* 11, 193 (1990).
- L. M. Muul, P. J. Spiess, E. P. Director, S. A. Rosenberg, *J. Immunol.* 138, 989 (1987).
- J. T. George *et al.*, *Cancer Res.* 53, 2374 (1993).
- B. E. Eliot, D. A. Carlow, A. Rodricks, A. Wade, *Adv. Cancer Res.* 53, 181 (1989).
- N. P. Restifo *et al.*, *J. Exp. Med.* 177, 265 (1993).
- L. Chen *et al.*, *Cell* 71, 1093 (1992); S. E. Townsend and J. P. Allison, *Science* 259, 368 (1993).
- J. Trojan *et al.*, *Science* 259, 94 (1993).
- R. W. Chestnut and H. M. Grey, *J. Immunol.* 128, 1075 (1981); A. Lanzavecchia, *Immunol. Rev.* 8, 773 (1990).
- Y. J. Guo *et al.*, *J. Mil. Med. Coll. People's Liberation Army* 3, 272 (1988).
- We prepared polyclonal antisera by injecting rabbits subcutaneously with BERH-2 hepatoma cells or purified activated B cells from Wistar rats, each mixed with Freund's complete adjuvant. Activated B cells were purified by panning with plates coated with purified goat antibody to rat immunoglobulin (IgG). After repeated boosting for 2 months, the antisera were collected and purified by passage over a protein G column. Finally, the antisera were repeatedly absorbed with either BERH-2 hepatoma cells or rat B cells.
- J. Alan and T. Robin, *Immunochemistry in Practice* (Blackwell, New York, ed. 2, 1988), chap. 2. Tumor cells were fused with activated B cells at a ratio of 1 tumor cell to 10 activated B cells. Fusions were carried out with 50% PEG in Dulbecco's phosphate-buffered saline without Ca<sup>2+</sup> or Mg<sup>2+</sup> at pH 7.4.
- Y. Guo *et al.*, unpublished results.
- E. R. Fearon *et al.*, *Cell* 60, 397 (1990); R. I. Tepper, P. K. Pattengale, P. Leder, *ibid.* 57, 503 (1989); T. Blankenstein *et al.*, *J. Exp. Med.* 173, 1047 (1991); Y. Watanabe *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 86, 9459 (1989).
- P. L. Linsley *et al.*, *J. Exp. Med.* 174, 561 (1991).
- Supported in part by a grant from The Science and Philosophy Betterment Society. Y.G. is a recipient of an International Exchange Research Award from the China Science & Nature Foundation. We thank the Bristol-Myers Squibb Pharmaceutical Research Institute for CTL4-Ig and B. Benacerraf, M. Bigby, S. Gerson, P. Lehmann, and M. Lamm for suggestions and reading of the manuscript.

14 September 1993; accepted 24 November 1993

## Parallel Neuronal Mechanisms for Short-Term Memory

Earl K. Miller\* and Robert Desimone

Although objects that have just been seen may persist in memory automatically for a time and interact passively with incoming stimulation, some tasks require that the memory be actively maintained and used. To test for the existence of separate automatic and volitional mechanisms of short-term memory, recordings were made from neurons in the inferior temporal cortex of monkeys while the monkeys held a sample picture "in mind" and signaled when it was repeated in a sequence of pictures, ignoring other stimulus repetitions. Some neurons were suppressed by any picture repetition, regardless of relevance, whereas others were enhanced, but only when a picture matched the sample. Short-term memory appears to reflect the parallel operation of these two mechanisms—one being automatic and the other active.

Combined evidence from psychology and neuroscience has cleaved long-term memory into two functionally independent systems: an explicit system for facts and events, and an implicit system for the learning of perceptual and motor skills and habits (1). Psychological studies suggest that there may be more than one neural system mediating short-term memory (STM) as well. Some theoretical accounts, for example, posit that incoming stimuli are automatically held in some type of short-term storage buffer but may, in addition, be voluntarily maintained by active rehearsal mechanisms (2). We sought neurophysiological evidence for multiple STM mechanisms in recordings from the anteroventral portion of the inferior temporal (IT) cortex, a region important for visual memory in primates, including humans (3).

Nearly all behavioral and physiological studies of memory in the IT cortex have used some variation of the delayed matching-to-sample (DMS) task, in which the subject indicates whether a test stimulus matches a previously shown sample stimulus. The

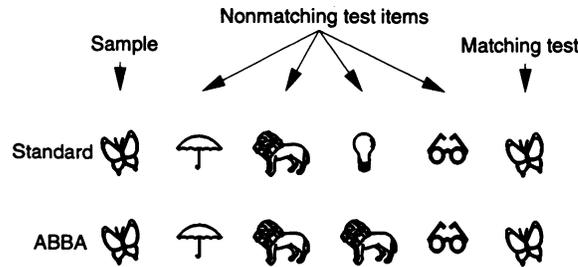
memory of the sample has a lasting effect on many IT neurons, because their response to subsequent test items is suppressed according to how well they match the sample—a property we have termed "adaptive mnemonic filtering" (4, 5). Because the sample is behaviorally relevant in DMS tasks, it is commonly assumed that it is actively maintained in memory (that is, "working memory"), interacting with the neural processing of incoming test stimuli; however, it is also possible that all stimuli, relevant or not (including, but not limited to, the sample), automatically linger in memory for a time, interacting with incoming stimuli. For example, if one actively searches for a repetition of the sample number 3897 in the following series—1436 3482 3482 3897—one may automatically detect the repeated but irrelevant number 3482, in addition to detecting the specific repetition of the sample number. Thus, detection of stimulus repetition in DMS tasks might be mediated by either automatic or active mnemonic mechanisms, or both.

To distinguish among these possibilities, we tested two monkeys with two types of trials (Fig. 1). The first type, standard trials, were conventional DMS trials identical to those used in our previous studies of adaptive

Laboratory of Neuropsychology, Building 49, Room 1B80, National Institute of Mental Health, Bethesda, MD 20892.

\*To whom correspondence should be addressed.

**Fig. 1.** A standard trial is illustrated in the top row and an ABBA trial in the bottom row. From zero to three test stimuli intervened between the sample and the final match. The repeated nonmatches appeared after either zero (pictured) or one intervening stimulus between them (for example, AB-CBA).



mnemonic filtering (4, 5). A sample stimulus (“A”) was followed by one or more sequential test stimuli (“BCDEA”), and the monkey was rewarded for signaling when one matched the sample (6). None of the test stimuli matched each other—the only repeated stimulus in the trial was the sample-match stimulus. The second type of trial was termed an “ABBA” trial. In these trials, two of the intervening nonmatch test stimuli (“BB”) matched each other but not the sample. The animal had to withhold its response to these repeated nonmatch stimuli and respond only to the repeated stimulus that matched the sample. Although the standard trials might be solved by a mecha-

nism that automatically detects any type of stimulus repetition regardless of relevance, the ABBA trials would force the animals to maintain the sample item in working memory and compare test items to it.

Both monkeys were originally trained with standard trials only. When their performance was 85 to 90% correct, the ABBA trials were introduced, comprising about half the total trials in a session and randomly intermingled with standard trials. Unexpectedly, initial performance on the ABBA trials revealed that the animals had learned standard DMS by using a simple stimulus-repetition rule rather than by comparing test stimuli to just the sample memory. That is, both monkeys released the bar to the repeated nonmatch stimuli (for example, to the second “B”), resulting in an error. In the first five sessions, performance on ABBA trials ranged from 0.5% (monkey 1) to 56% (monkey 2) correct, which was significantly worse than on the standard trials, which ranged from 85 to 90% correct, respectively (paired *t* test, monkey 1:  $t = 9.716$ ,  $P = 0.001$ ; monkey 2:  $t = 6.297$ ,  $P = 0.003$ ).

After 2 to 6 weeks of additional training, their performance on ABBA trials reached 85% correct. The animals now knew both to maintain the sample in memory and to compare test stimuli to it, and they presumably applied this new strategy to all trials,

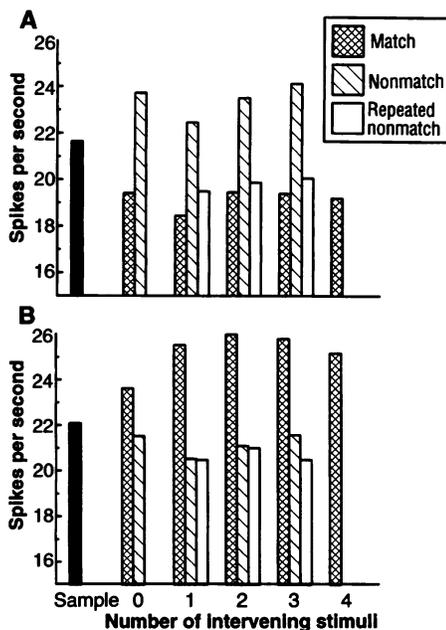
because they could not predict in advance whether a trial would be ABBA or standard. We then recorded from 148 IT neurons during standard and ABBA trials, randomly intermixed (7).

We first separated cells into mnemonic and nonmnemonic classes, with a two-way analysis of variance (ANOVA) carried out separately on each cell, evaluated at  $P < 0.05$ . The six stimuli were one factor and their matching-nonmatching status on a given trial was the other (8). The ANOVA showed that half the cells (74 out of 148) showed significant memory effects and that nearly all of these (70 out of 74) were also stimulus-selective. That is, the responses of these cells were a joint function of the current stimulus and of memory traces, which is consistent with previous findings (4, 5, 9, 10). Many cells also showed stimulus-specific activity in the delay following the sample (11), but this activity was abolished by the first intervening test stimulus. Useful mnemonic information was carried only in the cells’ responses to test items.

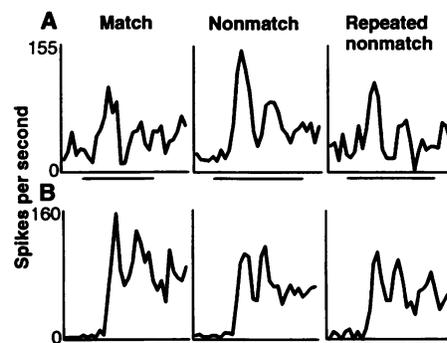
Of the cells showing memory effects, the responses of 62% (46 out of 74) were suppressed by test stimuli that matched the sample (as compared to nonmatch responses). Responses were suppressed even when up to three stimuli, the maximum tested, intervened between the sample and the matching stimulus, according to a paired *t* test ( $P < 0.001$ ) performed on the population data. This is the same “adaptive mnemonic filtering” found in previous studies (4, 5). For convenience, we will refer to it here as “match suppression.”

However, the ABBA trials revealed that the responses of these cells were suppressed not only by match stimuli but also by repeated nonmatches (Fig. 2A). Responses to matches and repeated nonmatches were not significantly different (paired *t* tests,  $P > 0.11$ ). The responses of one such cell are shown in Fig. 3A. Thus, responses were suppressed by both relevant and irrelevant stimulus repetitions within the trial, not just by the test stimulus that was a repetition (match) of the sample. These results suggest that adaptive mnemonic filtering underlies automatic memory for stimulus repetition.

In contrast to the suppressed cells, 35% (26 out of 74) of the cells with significant memory effects gave enhanced responses to test stimuli that matched the sample memory, as compared to nonmatching responses. We term this effect “match enhancement.” Although the suppressed cells did not distinguish between matches and repeated nonmatches, the responses of these enhanced cells were enhanced only by stimuli that matched the sample, not by the repeated nonmatch stimuli that “matched” each other (Fig. 2B). The responses of one such cell are shown in Fig. 3B. Like the suppression



**Fig. 2.** Average responses of suppressed and enhanced neurons. (A) Average response of 46 suppressed neurons to all 73 stimuli that elicited a significantly weaker response when they matched the sample than when they did not. Average responses (and SEM) to matches, nonmatches, and repeated nonmatches were 19.2 (0.8), 23.5 (1.0), and 19.8 (1.0) spikes per second, respectively. (B) Average response of 26 enhanced neurons to all 45 stimuli that elicited a significantly stronger response. Average responses (and SEM) to matches, nonmatches, and repeated nonmatches were 25.3 (1.5), 21.2 (1.6), and 20.7 (1.7) spikes per second, respectively. Spontaneous firing was approximately 10 spikes per second for both types of cells.



**Fig. 3.** (A) Responses of a suppressed neuron to a single stimulus appearing as a match, nonmatch, and repeated nonmatch. (B) Same as in (A), for an enhanced neuron. Horizontal bars under the histograms indicate when the stimuli were on, which was 500 ms for the nonmatch and repeated nonmatch stimuli. The match stimulus was terminated when the animal made its response ( $\beta$ ). The bin width is 20 ms.

Downloaded from www.sciencemag.org on August 14, 2013

effect, the enhancement effect lasts at least several seconds, as it was maintained even when three stimuli intervened between the sample and the final match, the maximum tested (paired *t* tests,  $P < 0.001$ ). The enhancement effect, like the animal itself, uniquely identified the one stimulus in the sequence that matched the actively maintained sample memory. This is consistent with an active, or working, memory mechanism. Only 3% of the cells (2 out of 74) showed mixed effects, namely suppression by some stimuli and enhancement by others. The two classes of cells appear to be distinct.

We asked whether the enhancement effect might be related to the behavioral response itself or to the expectation of reward. To address this question, we first examined response histograms averaged across the population of cells (Fig. 4). Match enhancement (Fig. 4B) and suppression effects (Fig. 4A) both occurred early in the visually evoked response of the neurons, about 80 to 90 ms after stimulus onset and well before the animals' mean behavioral response latency of 369 ms (range, 321 to 501 ms). Enhancement occurred nearly simultaneously with the arrival of visual information in the IT cortex, almost certainly before the animal chose the appropriate behavioral response.

We also examined responses of IT neurons to repeated nonmatches in error trials in which the animals released the bar to the repeated nonmatch. If enhancement were a result of the behavioral response and reward, the neurons' responses to repeated nonmatches should have been enhanced when the animals mistakenly responded to them. In fact, across the population of enhanced cells, responses to nonmatches (22.56 spikes per second) and to repeated nonmatches followed by an incorrect behavioral response (22.16 spikes per second) were not significantly different (paired *t* test,  $P = 0.79$ ). Enhancement is exclusively related to the memory aspect of the task.

In previous studies using animals trained only with standard trials, we found about the same proportion of cells with significant memory effects (48%) as in the present study, but the proportion of those cells showing match enhancement was significantly smaller than that found here (9% versus 35%,  $P = 0.0001$ ,  $\chi^2$ ) and the proportion showing suppression was correspondingly higher (4, 5). Furthermore, we recorded from 57 neurons in the same region of the cortex of one of the animals in our study before the animal had received ABBA training, and all cells with memory effects (40% of total cells) showed suppression. Although not yet conclusive, the relatively large number of enhanced cells we observed suggests that learning a new strategy in order to perform the ABBA trials resulted in a shift toward the enhancement mechanism.

Our results show that the IT cortex contains at least two mechanisms operating in parallel that could mediate visual STM (12). One, the suppression (or adaptive filtering) mechanism, is engaged by simple stimulus repetition. As a result, neuronal responses are largest for stimuli that differ from those seen in the immediate past, an effect that might be caused by activation-induced depression of synapses on IT cells. Such a mechanism might underlie the performance of standard DMS tasks used in behavioral and physiological studies and may explain automatic stimulus repetition effects in studies of human STM (2).

The second mechanism, enhancement, is engaged when primates deliberately use the contents of their working memory to search for a particular object occurring at an unknown time. In this case, IT neurons can be biased, or preset, to give a potentiated response to the expected stimulus. Because we have recently found IT-like enhancement and suppression effects in the prefrontal cortex (13), another region implicated in STM, these active and passive memory

mechanisms appear to be common components of distributed STM systems.

## REFERENCES AND NOTES

1. L. R. Squire, *Psychol. Rev.* **99**, 195 (1992).
2. A. Baddeley, *Working Memory* (Clarendon Press, Oxford, 1986).
3. J. A. Horel, D. E. Pytko-Joiner, M. L. Voytko, K. Salsbury, *Behav. Brain Res.* **23**, 29 (1987); S. Zola-Morgan, L. R. Squire, D. G. Amaral, W. A. Suzuki, *J. Neurosci.* **9**, 4355 (1989); D. Gaffan and E. Murray, *Behav. Neurosci.* **106**, 30 (1992); M. Meunier, J. Bachevalier, M. Mishkin, E. A. Murray, *J. Neurosci.* **13**, 5419 (1993).
4. E. K. Miller, L. Li, R. Desimone, *Science* **254**, 1377 (1991).
5. ———, *J. Neurosci.* **13**, 1460 (1993).
6. Two rhesus monkeys were trained to fixate a small target on a computer display over which a series of stimuli was presented sequentially. Stimuli were digitized pictures of complex objects, 1° to 3° wide, presented for 500 ms and separated by 1000-ms intervals. The stimuli were chosen randomly for each neuron tested and were shown repeatedly before data collection began so that they would be familiar to the monkeys. When a test stimulus matched a sample stimulus, the monkey released a bar for a reward of juice, terminating the stimulus. Fixation was monitored by a magnetic search coil. For details, see (5).
7. Electrode sites were localized between the anterior middle temporal and rhinal sulci in the IT cortex, by magnetic resonance imaging in both monkeys, with histological verification in one.
8. Neuronal responses were averaged over the 75- to 275-ms interval immediately after stimulus onset, ending well before the animals' behavioral response to the match stimulus. For the ANOVA, we excluded responses to matches that appeared after four intervening stimuli, so that the mean number of intervening items preceding matches and nonmatches was the same. We also excluded responses to repeated nonmatches so that these responses could later be examined independently.
9. Other studies of IT neurons using standard DMS tasks have likewise found significant suppression effects almost exclusively [C. G. Gross, D. B. Bender, G. L. Gerstein, *Neuropsychologia* **17**, 215 (1979); G. C. Baylis and E. T. Rolls, *Exp. Brain Res.* **65**, 614 (1987); I. P. Riches, F. A. Wilson, M. W. Brown, *J. Neurosci.* **11**, 1763 (1991); E. N. Eskandar, B. J. Richmond, L. M. Optican, *J. Neurophysiol.* **68**, 1277 (1992)].
10. Neither the stimulus nor the memory had an "all-or-none effect" on the cells' responses. Rather, whether or not the current stimulus matched the memory trace caused a modulation of the underlying sensory response, which is consistent with the results of prior studies (4, 5, 9).
11. J. M. Fuster and J. P. Jervey, *Science* **212**, 952 (1981).
12. Because neither the suppression nor the enhancement bridged stimuli across trials when the stimuli were already familiar, the effects are within the domain of STM [see (4, 5) for the relevant tests of the suppression mechanism].
13. L. Chelazzi, E. K. Miller, A. Lueschow, R. Desimone, *Soc. Neurosci. Abstr.* **19**, 975 (1993). The prefrontal cortex may also be the source of the bias signal for enhanced cells, because prefrontal cells show stimulus-specific maintained activity during the delay interval of delayed response tasks [J. M. Fuster, R. H. Bauer, J. P. Jervey, *Exp. Neurol.* **77**, 679 (1982); F. A. W. Wilson, S. P. O. Scalaidhe, P. S. Goldman-Rakic, *Science* **260**, 1955 (1993)]. We have recently found that this maintained activity survives intervening items in the ABBA task, unlike in the IT cortex.
14. We thank L. Chelazzi, P. DeWeerd, A. Lueschow, M. Mishkin, J. I. Nelson, and L. G. Ungerleider for comments on the manuscript and T. White for computer programming. Supported in part by the Human Frontiers Science Program Organization.

4 August 1993; accepted 30 November 1993

**Fig. 4.** (A) Population average histogram for suppressed neurons responding to the same stimuli as in Fig. 2A. (B) Population average histogram for enhanced neurons responding to the same stimuli as in Fig. 2B. See Fig. 3 for conventions.

