vectors (2, 5) that encode Ub–X–βgals downstream from a galactose-inducible (GAL10) yeast promoter. This promoter functions as a weak constitutive promoter in E. coli (10). The yeast Ub-specific protease Ubp1 was expressed in E. coli from the plasmid pJT184, whose P15A replicon is compatible with that of pUB23 (2, 10).

12. Escherichia coli BW13711 [F⁻λ⁻K12 Δ(lac)ₓ₇₄] [W. W. Metcalf, P. M. Steed, B. L. Wanner, J. Bacteriol. 172, 3191 (1990)] carrying pUB23-X, which expressed Ub–X–βgal, and pJT184, which expressed Ubp1 (11), were grown at 36°C to an optical density at 600 nm of ~0.3 in M9 medium supplemented as described (10). Cells in 5 ml of the culture were collected by centrifugation, resuspended in M9 medium (0.25 ml), and incubated at 36°C for 10 min. [³⁵S]Translabel (ICN Biomedicals) (0.1 mCi) was then added, and cells were incubated for 2 min. Chase solution (12.5 μl) containing L-Met (40 mg/ml) and L-Cys (10 mg/ml) was added, and a portion of the cell suspension (50 μl) was withdrawn, transferred into a chilled tube containing lysis buffer (50 μl) [4% SDS and 125 mM tris-HCl, (pH 6.8)], and frozen by immersion in liquid N₂. The rest of the cell suspension was incubated at 36°C with intermittent mixing; aliquots (50 μl) were withdrawn at various times after the pulse and processed as above. The chase points in Fig. 1A were 3, 10, 30, and 60 min for long-lived X-βgals and 3, 5, 10, and 30 min for short-lived X-βgals. Conditions of the pulse-chase assay did not perturb the N-end rule pathway because cells incubated under these conditions for 1 hour before the pulse labeling did not alter kinetics of X-βgal degradation. The frozen samples were heated at 100°C for 4 min. Each sample was then added to immunoprecipitation (IP) buffer (1 ml) [1% Triton X-100, 0.5% deoxycholate, 0.15 M NaCl, 5 mM EDTA, 50 mM Hepes, (pH 7.5), plus freshly added 1 mM phenylmethylsulfonyl fluoride] and centrifuged at 12,000g for 10 min at 4°C. The supernatant (0.9 ml) was added to a solution (1 μl) containing a monoclonal antibody to βgal (2 mg/ml; Promega) and incubated at 0°C for 1 hour. A protein A–agarose suspension (Repligen) (10 μl) was then added; the suspension was incubated with rocking for 30 min at 4°C and centrifuged for 15 s at 12,000g. The pellet was washed three times with IP buffer containing 0.1% SDS, dissolved in SDS-containing sample buffer, and subjected to electrophoresis in a SDS–6% polyacrylamide gel (10). The gels were washed in 25% CH₃OH and 10% CH₃COOH for 20 min, dried, autoradiographed, and quantified (22).

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A Neural Mechanism for Working and Recognition Memory in Inferior Temporal Cortex

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Inferior temporal (IT) cortex is critical for visual memory, but it is not known how IT neurons retain memories while new information is streaming into the visual system. Single neurons were therefore recorded from IT cortex of two rhesus monkeys performing tasks that required them to hold items in memory while concurrently viewing other items. The neuronal response to an incoming visual stimulus was attenuated if it matched a stimulus actively held in working memory, even when several other stimuli intervened. The neuronal response to novel stimuli declined as the stimuli became familiar to the animal. IT neurons appear to function as adaptive mnemonic "filters" that preferentially pass information about new, unexpected, or not recently seen stimuli.

THE IT CORTEX OF BOTH HUMAN and nonhuman primates is critical for visual memory. In monkeys, removal of IT cortex impairs performance of tasks that require judgments of either stimulus recency (working memory) or stimulus familiarity (recognition memory). In the absence of IT cortex, the memory of a visual stimulus decays significantly over the course of a minute or two and may decay even more rapidly if the subject views other stimuli during the retention interval (1). It is not understood how memories for stimuli are retained in IT cortex, particularly as new stimuli continuously enter the visual system and, presumably, activate the same IT neurons involved in the retention of memories.

We therefore recorded from neurons in the anterior IT cortex of two rhesus monkeys that were required to retain items in memory while viewing a series of other stimuli (2). We used two variants of a delayed matching-to-sample task designed to explore the role of IT neurons in either working or recognition memory. On each trial, while the monkey held a bar and maintained fixation on a fixation target (3), a sample stimulus was presented at the center of gaze followed by sequential presentation of one to five test stimuli. All sample and test stimuli were on for 500 ms each, separated by 700-ms delays. The monkey held onto the bar until one of the test stimuli in the sequence matched the sample, at which point it released the bar for an orange juice reward, terminating the trial (4). The nonmatching stimuli that intervened between the sample and final matching stimulus differed from each other and from the sample. The stimuli were color-digitized, common objects presented on a computer graphics display.

In the working memory procedure, we asked whether the particular sample stimulus the animal held in memory on each trial affected how cells processed new inputs (the test stimuli). Each cell was studied with six familiar stimuli, each of which appeared as the sample and matching stimulus on some trials, and as a nonmatching stimulus on others, for a total of about 360 trials per cell (5).

We initially recorded from 146 IT neurons and found that nearly half responded selectively not only to particular test items but also according to whether the test items matched the sample item held in memory. Two-way analyses of variance (ANOVAs) were applied to the responses of each cell separately, with the six test items and the matching-nonmatching status as factors. The responses to matching stimuli that appeared after four intervening items were excluded from all statistical analyses, so that the mean number of intervening items preceding matching and nonmatching stimuli

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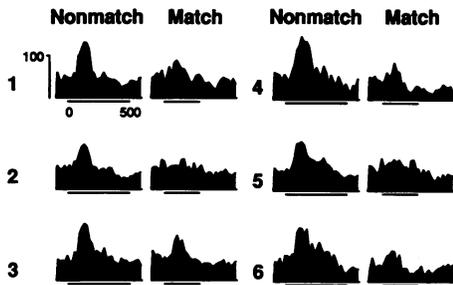
Fig. 1. Responses of one IT neuron to six test items, which matched the sample held in memory on some trials and did not match on others. Each histogram represents the firing rate to a stimulus averaged over approximately 30 trials, with one to four presentations of intervening stimuli per trial. Horizontal bars under the histograms indicate the period during which the stimuli were on. Nonmatching stimuli were on for 500 ms. Matching stimuli were turned off when the monkey made its response, about 350 ms after stimulus onset. The vertical bar indicates a firing rate of 100 spikes per second.

was the same. On the basis of the ANOVAs (evaluated at $P < 0.05$), 85% of the cells were stimulus-selective, independent of whether the test items were matching or nonmatching, 48% of the cells distinguished between matching and nonmatching items, independent of stimulus selectivity, and 15% showed a significant interaction between these two factors. The latter cells commonly showed a disproportionately large matching-nonmatching effect for their "optimal" stimulus.

For nearly all cells that responded differently to matching and nonmatching stimuli, the response to matching stimuli was smaller than to nonmatching (Fig. 1). This response attenuation to matching stimuli was apparent even when up to four nonmatching stimuli (the maximum tested) intervened (Fig. 2A). Control experiments showed that neither the behavioral response to the matching stimulus nor the expectation of reward was necessary to obtain the response attenuation. Furthermore, a comparison of responses to the same sample stimulus presented on successive trials indicated that the response attenuation was caused not solely by the temporal contiguity of the sample and match but rather by a more active matching process that was reset between trials. Thus, the responses of IT neurons could underlie the ability to perform working memory tasks spanning multiple stimulus items. Prior studies of IT cortex either did not test for such mnemonic effects spanning intervening items or failed to find such evidence when they did (6, 7).

The difference in response to matching and nonmatching stimuli tended to decrease with increasing numbers of intervening items (Fig. 2A). The trend suggested that the difference might be abolished if any additional items

intervened. To test this, we studied an additional 18 cells in a more difficult version of the task, in which up to six stimuli intervened between the sample and matching stimulus. When the animal was trained and subsequently tested on the more difficult task, the difference in response to matching and nonmatching stimuli was now maintained after the additional intervening items (Fig. 2B). This result suggests that there may be no limit on the "memory span" of IT neurons, as long as the animal retains the sample item in memory.

As a measure of how much information about the remembered sample was carried in the neuronal responses to the test items, we asked how well one could classify a stimulus as matching or nonmatching on any given trial based on the response of an individual cell. To make such a classification, one needs to know the function relating response magnitude to the probability that a given stimulus is matching or nonmatching. Using logistic regression (8), we estimated this function separately for each stimulus and each cell. The regression was significant (likelihood ratio test, $P < 0.05$) for 27% of the stimuli that elicited any response. For each of these stimuli, we then used the regression equation as a decision rule to predict, for each response, whether a stimulus was matching or nonmatching. If there was a perfect relationship between firing rate and matching-nonmatching status, the success rate should have been 100%, whereas any response variability would reduce the success rate below that level. We compared the prediction to the actual stimulus and
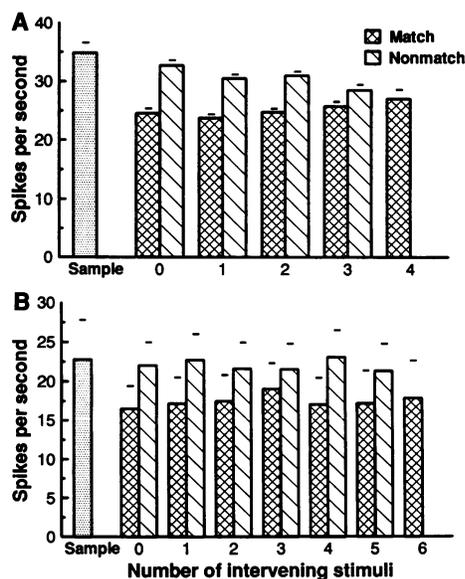
Fig. 2. (A) Average response (and SEM) of 146 cells to all 171 test items that showed a significant matching versus nonmatching response difference. (B) Same as in (A) but for 18 additional cells studied with up to six intervening items.
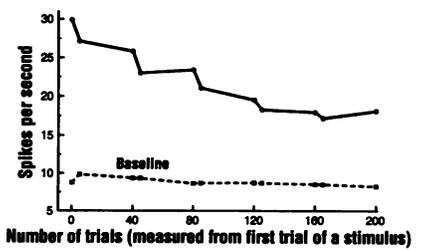
Fig. 3. Average response of 25 IT neurons that showed a significant attenuation of response across the test session to initially novel stimuli. Responses were averaged across all stimuli and all cells, and the trial number for a given stimulus was measured from the first trial on which that stimulus appeared. The baseline is the average spontaneous firing rate of the cells, measured before each trial.

found a mean successful classification rate of 59% correct (chance, 50%), with a range of 52 to 71%. Although no individual neuron performed as well in the task as the animal as a whole, in principle one could achieve a success rate equal to that of the animals' (90% correct) by averaging the responses of just a few IT cells to reduce the response variability.

It was not only the response to matching stimuli that carried information about the specific sample item held in memory but also the responses to nonmatching stimuli. We applied the logistic regression analysis to the responses to the nonmatching items alone, using the regression equation to predict which of the five possible samples preceded a given nonmatching item on each trial (excluding the one sample that matched the test item, out of the six possible). The regression was significant for 25% of the nonmatching items that elicited responses; for these items, the mean success in classifying which sample had been used on a given trial was 29% (chance, 20%), with a range of 22 to 40%. This effect of the samples on responses to nonmatching items would be explained if the responses to all incoming test items, matching as well as nonmatching, were attenuated according to their similarity to the sample. That is, it may be the degree of similarity of the current stimulus to the memory trace that causes the attenuation of response, rather than matching per se.

In the recognition memory procedure, we asked how the neuronal responses to a stimulus the animal had never seen before changed over the course of the recording session, as the stimulus gradually became more familiar. Although the basic task was the same as in the working memory test, our interest was in the incidental memories that would accrue as the animal repeatedly experienced the same initially novel stimuli over the course of the session. Each cell was studied with 20 novel stimuli, with each appearing as the sample and match, but not

as the nonmatch. A new set of novel stimuli was used for each cell, and a given set was reused, if at all, no sooner than several months later. The nonmatching items used on each trial were chosen from a set of four different, already highly familiar, stimuli. A given sample stimulus was repeated in the session after 4 or 35 intervening trials with the other sample stimuli, the 4- and 35-trial intervals being used in alternation. About 200 to 400 trials, or 10 to 20 trials per sample stimulus, were typically used in each session.

For many IT neurons, the response to the initially novel sample stimuli systematically declined over the session as the animal gained more experience with them. Over one-third of the 72 neurons tested exhibited a significant decrease in response with repeated presentation of these stimuli (9) (Fig. 3), the amount of the decrement increasing across trials, reaching a stable level (still above the spontaneous firing rate) after about six to eight trials. The largest decrements were typically found for stimuli that caused the greatest initial response. A parallel decline during the session was seen for responses to the same stimuli presented as the matching item at the end of each trial. For the remaining neurons, there was either no change in response across the session or, infrequently (9%), a small increase in response. Because the number of cells responding strongly to an initially novel stimulus was smaller at the end of the session than at the beginning, there appears to be a focusing of activation within the population of cells as a result of experience.

The magnitude of the decrease in response to a given sample on a given trial depended on the number of intervening trials between successive presentations of the same sample. The decrement was much larger when only 4 trials intervened than when 35 trials intervened, although the cumulative decrement was retained even after 35 trials. Simple fatigue predicted the opposite, that is, greater decrements in response with the greater number of intervening trials, as the cells were activated on the intervening trials by many other stimuli. Thus, the cells could apparently detect that a specific stimulus had been presented before in the session, even after 4 to 35 intervening trials, or 16 to 140 intervening stimuli (given an average of four stimuli per trial). Consistent with this conclusion, when a few cells were retested with a new stimulus set, they showed a recovery of response on the initial presentation of the new stimuli. We do not yet know whether the decline in response to familiar stimuli is maintained for days (10).

Our results show that the responses of IT neurons to incoming visual stimuli carry information about the memories of past stimuli. The responses appear to be modulated according to the similarity of the stimulus to memory traces, whether the trace is actively held in short-term working memory or is passively held in a longer term store. The formation of memories may consist, at least in part, of the modification of synaptic weights such that familiar, expected, or recently seen stimuli cause the least activation of the cortex. This seemingly counterintuitive view is consistent with some neural network models of memory as well as with recent findings in humans and monkeys (7, 11). IT neurons may be acting as adaptive mnemonic filters that seek to preferentially pass information about new, unexpected, or not recently seen stimuli. Such a process would not simply precede memory storage but would be a critical component of the storage mechanism.

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Lateral Movements of Membrane Glycoproteins Restricted by Dynamic Cytoplasmic Barriers

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Cell membranes often are patchy, composed of lateral domains. These domains may be formed by barriers within or on either side of the membrane bilayer. Major histocompatibility complex (MHC) class 1 molecules that were either transmembrane- (H-2D^b) or glycosylphosphatidylinositol (GPI)-anchored (Qa2) were labeled with antibody-coated gold particles and moved across the cell surface with a laser optical tweezers until they encountered a barrier, the barrier-free path length (BFP). At room temperature, the BFPs of Qa2 and H-2D^b were 1.7 ± 0.2 and 0.6 ± 0.1 (micrometers ± SEM), respectively. Barriers persisted at 34°C, although the BFP for both MHC molecules was fivefold greater at 34°C than at 23°C. This indicates that barriers to lateral movement are primarily on the cytoplasmic half of the membrane and are dynamic.

ALTHOUGH THE LATERAL DIFFUsion of a few membrane proteins, notably visual rhodopsin, is rapid and appears to be hindered only by the viscosity of membrane lipids, the lateral diffusion of most proteins is hindered in several ways. Significant fractions of most membrane proteins are immobile, and diffusion coefficients for the mobile fractions are 10- to 100-fold lower than that of rhodopsin (1). In erythrocyte membranes, the spectrin-actin complex limits the lateral diffusion of band 3 (micrometer scale) (2) but has little effect on rotation of band 3

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