Inferotemporal Neurons Distinguish and Retain Behaviorally Relevant Features of Visual Stimuli

Abstract. *Single-cell activity was recorded in the inferotemporal cortex of monkeys performing a task that requires perception and temporary retention of colored stimuli. Many cells reacted differentially to the stimuli. By changing the relevance of certain features of compound stimuli, it was found that the reactions of some cells to color depend critically on whether or not the task demands that the animal pay attention to color. A substantial number of cells showed color-dependent differences in frequency of discharge during the retention periods of the task. The temporal characteristics of differential discharge and its dissolution when memory is no longer required indicate that the cells that display it are involved in retaining visual information.*

In primates, the inferior temporal cortex—area TE (*1*)—is involved in vision (*2*). Ablation and reversible-lesion studies have implicated that cortex in visual discrimination (*3*), visual attention (*4, 5*), and visual short-term memory (*4, 6, 7*). Single-unit studies have revealed visual properties of inferotemporal (IT) cells—large receptive fields including the fovea and selective reactivity to such stimulus dimensions as shape, orientation, and color (*8*). Unit recording in behaving animals has shown that the reactions of IT cells to photic stimuli are subject to modulation by attention and situational variables (*9, 10*). However, lesion and unit studies thus far have not delineated precisely the role of IT cortex in visual information processing. Attempting to elucidate this matter, we explored the activity of IT cells in monkeys during performance of a task requiring perception, temporary memory, and recognition of visual stimuli. We focused in particular on IT cell discharge during attention to a discrete stimulus and during the subsequent retention of that stimulus. Some cells not only reacted differently to different colors—the task memorandum—but reacted differently to a single color depending on whether or not the animal had to attend to it in order to meet task demands. During retention periods, some cells discharged at different rates depending on the color of the stimulus that the animal had to retain to meet those demands.

Our findings are based on the study of more than 500 cells in the temporal cortex of nine macaques trained to perform a delayed matching-to-sample task. An experimental animal sat in a primate chair facing a white panel with translucent stimulus-response buttons (diameter 2.5 cm) that, by rear projection, could display colors or geometric symbols. A trial consisted of the following sequence: (i) presentation of the sample stimulus—a color or a combination of color and symbol—in a central button at eye level (*11*); (ii) termination of the stimulus by the monkey's pressing the button; (iii) a delay, varying between 6 and 32 seconds (ordinarily about 18 seconds in recording sessions); (iv) simultaneous appearance of two or four stimuli, one of them the sample, in a horizontal array of buttons under the sample button; (v) monkey's choice of one stimulus by pressing the button that displayed it; and (vi) juice reward delivered to the monkey's mouth if the chosen stimulus matched the sample. The sample and its position in the choice buttons were changed randomly. Thus, for a correct response not attributable to chance, the animal had to perceive and remember the sample. Five monkeys performed the task with four colors: red, yellow, green, and blue; two monkeys, with two colors: red and green. The other two monkeys performed with color-symbol combinations: the sample consisted of a gray symbol (=, ○, or X) on a red or green background; if the sample contained the symbol =, the animal had to remember the color, for he was later presented with both colors, each with = in it, and had to choose the sample color for reward; if the sample contained one of the other two symbols (○ or X), the animal had to retain the symbol and ignore the color, for later he had to choose between the two symbols on a white background.

Hollow pedestals for a microelectrode positioner (*12*) were surgically implanted in the temporal bone of each fully trained monkey under Nembutal anesthesia. Metal sockets for head fixation during recording were attached to the skull with acrylic. Two animals were fitted with periorbital Ag-AgCl electrodes for recording eye movements [electrooculogram (EOG)]. Recording sessions were initiated several days after full recovery from surgery. Single-unit spikes were extracellularly recorded with metal microelectrodes driven through IT cortex during task performance. At the end of the experiments, small electrolytic lesions were made to mark electrode tracts and positions. After death, the animal's brain was fixed in Formalin and cut in 80-μm sections. The position of recorded

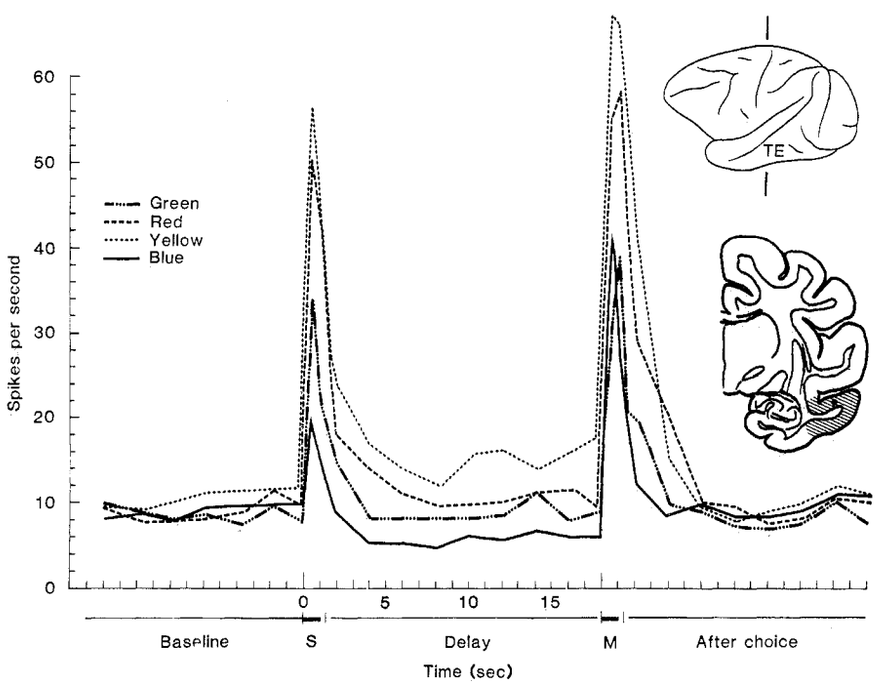

Fig. 1. Mean spike frequency of a cell in the IT cortex during delayed matching to sample with four colors. Sample (*S*) and matching (*M*) periods are marked with horizontal bars below the time axis. Every frequency curve represents average firing for four to six trials with each of the four sample colors. Note the differential reaction to the sample colors (maximum to yellow and minimum to blue), color-dependent differences in activity during 17-second delays, and after reactivation at the match, immediate return to baseline levels. The brain diagrams at the right show the position of the cell (dot) in the cortex of area TE (shaded in cross section) lining the lower bank of the superior temporal sulcus.

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units was reconstructed on photographic enlargements of the Nissl-stained sections. Unit activity was statistically analyzed and graphically displayed with a MINC-11 computer.

All cells selected for study exhibited changes of firing frequency in some phase of delayed-matching trials (the criterion for selection). Most of the cells in the IT cortex (inferior temporal convexity and lower bank of the superior temporal sulcus) responded with increased firing to one or more of the sample stimuli. About one-third of all IT units recorded showed differential reactions to the stimuli (Fig. 1 and Table 1), a few in the form of color-opponent changes. The latencies of change (after sample onset) were generally long (> 75 msec). About the same proportion of cells showed predominant activation by red as by green, the two sample colors uniformly used in all monkeys. Among the cells tested with color-symbol combinations, some reacted primarily to pattern and others to color regardless of relevance to the task. Others, however, reacted preferentially

Table 1. Cell response types in red and green sample trials of color-only delayed matching task. Percentages are given in parentheses.

Cortical region	Units recorded (No.)	Sample response			Delay activity change*		
		None	Color independent†	Color dependent‡	None	Color independent†	Color dependent‡
Superior temporal sulcus, upper bank	76	3 (4)	62 (82)	11 (14)	37 (49)	39 (51)	0 (0)
Superior temporal sulcus, lower bank	249	30 (12)	152 (61)	67 (27)	103 (41)	121 (49)	25 (10)
Inferior temporal convexity	110	6 (5)	62 (56)	42 (38)	63 (57)	42 (38)	5 (5)

*With respect to intertrial baseline. †Nondifferential excitation or inhibition. ‡Differential excitation or inhibition.

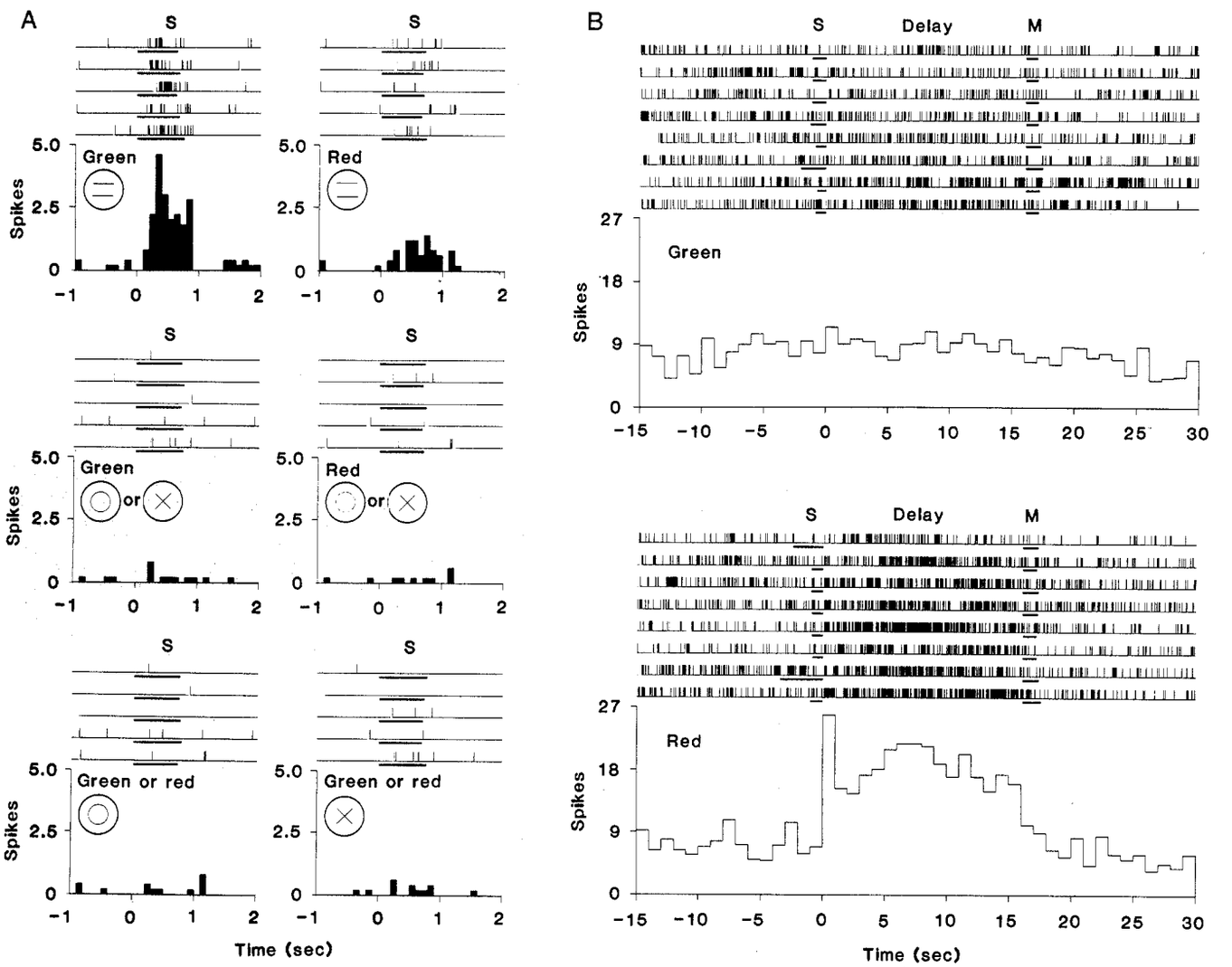

Fig. 2. (A) Spike activity of an IT cell in the sample period (*S*, horizontal bars) of delayed matching trials with color-symbol combinations. Each graph shows sample-period activity in five trials (each separately on top and in an average-frequency histogram below). Color was relevant when the sample contained the = sign, irrelevant when it contained either of the other two symbols. Note preferential activation by green, but only when color was relevant. (B) Discharge records and histograms from another IT cell during red and green trials. The histograms are time-locked with the end of the sample period (*S*) and the beginning of the match period (*M*). Note activation in delays after red and no appreciable changes in the sample period.

to one color only if the monkey had to attend to color for subsequent correct response at the end of delay (Fig. 2A).

In the cortex of the lower bank of the superior temporal sulcus were many cells showing sustained elevations of discharge during the delay. Trains of spikes at frequencies higher than intertrial baseline occupied the delay period in its entirety or in part. Although the duration and spike frequency of such trains varied considerably between trials, discharge, on the average, tended to diminish during the course of the delay. The delay activity of most cells was unrelated to any particular sample stimulus (Table 1). That of some, however, depended on the color of that stimulus (*13*). Not uncommonly, the color-related differences in firing of a given cell during delays reproduced comparable differences during sample periods (Fig. 1). However, neither changes of firing in the sample period nor the sample-specific character of such changes were necessary conditions for differential activity during delays (Fig. 2B).

A majority of cells activated at the sample were reactivated in the matching period. Cells activated only during matching were rarely encountered. Differential reactions at the sample were frequently followed by similar reactions at the match (Fig. 1).

A more precise description of cell types and their distribution will be published elsewhere (*14*). Cells with similar characteristics were often found in clusters; however, the configuration of such clusters could not be determined with confidence. Cells exhibiting delay activation, differential or nondifferential, appeared especially common in a rostral sector of the inferior bank of the superior temporal sulcus—areas IPA and TEa (*15*).

A decade ago, Gross *et al.* (*16*) speculated that the adequacy of visual stimuli for driving IT cells might depend on the significance of the stimuli as well as on their physical characteristics. Our results confirm that hypothesis. By manipulating the meaning of discrete features of compound stimuli, we have been able to demonstrate that at least some cells are attuned to the behavioral relevance of color.

For reasons that are not clear, Mikami and Kubota (*10*), using a somewhat similar delay-task (Konorski task), failed to find IT units with differential discharge during its intratrial delays. The discrepancy with our results may be due to differences of behavioral paradigm and to the brevity of the delays (1 to 5 seconds) used by those authors in a single-unit exploration that apparently did not extend into the depth of the superior temporal sulcus.

The protracted and differential discharge of IT cells during the delay is probably related to retention of the sample stimulus, that is, to the role of that stimulus as a memorandum. This inference is substantiated by evidence that differential firing was no longer observable after the trials ended, when the requirement of memorization was abrogated (*17*); sustained and color-dependent discharge usually did not continue or recur after trials (Figs. 1 and 2B), even though for a correct response, at the time of choice, the animal was obliged to foveate again the sample color, as confirmed by unit activity and EOG (*4, 14*).

Although the memory interpretation of delay activity is plausible and consistent with our data, we should consider the possibility that the sustained firing of at least some cells might be a kind of sensory afterdischarge following the sample stimulus (*18*). The concept of afterdischarge, however, would not easily apply to sustained delay activations in the absence of reaction to the sample or to the behavior of cells that show protracted excitation after one color and inhibition after another (Fig. 1).

If we accept the notion of a memory role for some IT cells, we should also consider that in our experiments the number of test colors (samples) was limited. Therefore, the preferential activation of one of those cells after a given color did not necessarily denote the specialization of the cell in retention of that particular color. Furthermore, in most cases, the observed color-dependent differences during the delay were simply differences in level of activation. This relativity of differences suggests that some cells participated in assemblies activated not only by color but by other features that the testing samples shared —size, location, shape, and brightness of the button. Only inasmuch as those other features were constant for stimuli and trials (as were the movements of the arm, the patterns of ocular motility, and the association with reward) may we tentatively conclude that color-related discharge during delays indicates participation of the cells that exhibit it in representation and retention of colors.

Insofar as delay-activated units concentrated in anterior IT cortex, our findings are in harmony with lesion studies. Such studies have suggested a functional dichotomy between posterior and anterior cortex, implicating the former in perception and the latter in a memory function (*7, 19*). However, our observations also hint at another, perhaps as important, dichotomy: one between cortex of the middle temporal gyrus and cortex in the lower bank of the superior temporal sulcus. In the first, units altered only during sample (and matching) periods were prevalent, and in the second, units that instead or in addition were altered during delays. Although our findings pertain to only a very restricted aspect of vision, they suggest that, after being subjected to perceptual analysis in the cortical IT convexity, visual information may be transferred to sulcal cortex for temporary storage. In any event, the functional gradients implied by the distribution of unit types from posterior to anterior cortex, and from convexity to sulcal cortex, follow the direction of some of the anatomical pathways thus far discovered within the IT cortex (*15, 20*).

The physiological involvement of IT cells in attentive and mnemonic processes, as indicated here, would explain the deleterious effects of IT lesions on the performance of visual discrimination and memory tasks (*3–7*). The patterns of unit activity we observed are probably expression of neural mechanisms fundamentally important for encoding, retaining, and retrieving visual data. Indeed, the formation of long-term visual memory may essentially depend on the proper functioning of those mechanisms, which are put to a test in the visual short-term memory task and which are reflected, during its performance, in the activity of the neurons of the inferior temporal cortex.

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11. The sample button subtended approximately 8° and was entirely transilluminated with colored light. Its luminance, with color-alone samples, was about 13.5 cd/m^2. The area covered by the symbol in color-symbol combinations was calculated to be the same for all symbols, such that the averaged luminance of the button, whatever the combination, was about 30 cd/m^2. Intensity and colorimetric characteristics were determined with a Pritchard Spectra photometer. The luminances of the sample stimuli used by any given animal were carefully equalized and periodically checked. Deviations from the mean for different colors or color-symbol combinations in no case exceeded 0.1 log unit. Wavelength was varied by the use of Cinemoid Color filters; dominant λ was about 620 nm for red, 590 nm for yellow, 530 nm for green, and 480 nm for blue.
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13. Very few cells with differential delay activity could be tested on color-symbol combinations. The results of this part of our experiments, which is continuing, are still inconclusive.
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17. Recording unit responses in untrained animals would not provide suitable control of the memory factor, since in such animals it is not possible to duplicate conditions of attention and perception of the sample stimulus as in the memory task.
18. In the acute preparation, some IT cells have been noted to react to visual stimuli with excitatory discharge that outlasts the stimulus by many seconds [C. G. Gross, personal communication; C. G. Gross, D. B. Bender, M. Mishkin, *Brain Res.* **131**, 227 (1977)].
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Peripherally Administered Reduced Pterins Do Enter the Brain

Abstract. *The content of tetrahydrobiopterin in rat brain was doubled by peripherally administered tetrahydrobiopterin, with the natural* l *diastereoisomer more effective than the unnatural* d *configuration. The model pteridine, 6-methyltetrahydropterin was ten times more efficient than tetrahydrobiopterin in crossing the blood-brain barrier, and striatal concentrations of 6-methyltetrahydropterin remained elevated for 2 hours, declining with a half-life of 3 hours. While no evidence for a specific uptake mechanism for concentrating 6-methyltetrahydropterin in cells containing tetrahydrobiopterin was detected, the pterin was found in its presumed site of action, the nerve terminal. Replacement therapy with reduced pterins may therefore be effective in the treatment of the neurological disorders associated with the variant forms of hyperphenylalaninemia that result from defects in the biosynthesis or metabolism of tetrahydrobiopterin within the central nervous system.*

A hyperphenylalaninemia in which neurological disorders persist despite dietary control of phenylalanine levels has been described (*1*). This condition, which is produced by defects in the phenylalanine hydroxylase system other than in phenylalanine hydroxylase itself, is characterized by a deficiency of dihydropteridine reductase (*2*) or by a defect in the biopterin biosynthetic pathway leading to a deficiency of tetrahydrobiopterin (BH_4) (*3*). Because the reductase and BH_4 are also necessary for the activity of tyrosine and tryptophan hydroxylases (*4*), this condition is further characterized by a lack of catecholamines and serotonin in the peripheral and central nervous systems (*5*). The variant forms of hyperphenylalaninemia are currently treated by restriction of phenylalanine intake and administration of dopa and 5-hydroxytryptophan, the hydroxylated amino acid precursors of catecholamines and serotonin, respectively, in conjunction with peripheral aromatic amino acid decarboxylase inhibition (*5*, *6*). Although administration of BH_4 might also appear to be a reasonable therapy (*2*), the reports that the blood-brain barrier is impermeable to peripherally administered BH_4 (*7*) made it seem unlikely that this treatment would prevent the neurological damage that characterizes these diseases. We now report that reduced pterins administered peripherally in relatively large doses do cross the blood-brain barrier and in a fashion that reflects the lipophobicity of the side-chain substitution at position 6 of the pterin ring.

Male Sprague-Dawley rats (150 to 200 g) were injected intraperitoneally with tetrahydropterins dissolved in 1 percent ascorbic acid, *p*H 7.0. Except where noted, animals received an overdose of barbiturate (500 mg/kg) 90 minutes later and were perfused through the heart with 50 ml of phosphate-buffered saline, *p*H 7.4, to flush the cerebral vasculature. Brains were removed, freed from membranes, rinsed in saline, dissected if necessary, and frozen at −70°C. Pterin content was determined by reverse-phase high-performance liquid chromatographic analysis with fluorescence detection (*8*).

Administered peripherally at a dose of 0.10 μmole per gram of body weight, BH_4 entered the brain in quantities which, although only 0.4 percent of what might have been expected on the assumption of equal body distribution, still were sufficient to increase whole-brain biopterin levels by a factor of 2 (Table 1). Analysis of the state of reduction of the accumulated pterin by differential oxidation demonstrated that more than 95 percent remained in the fully reduced, tetrahydro form (*8*).

The chemical reduction of biopterin introduces an asymmetric center at position 6 of the pterin ring, producing two diastereoisomers that can be resolved into the natural *l* and unnatural *d* isomers (*9*). Since the pterin-dependent monooxygenases exhibit different properties in the presence of the separate diastereoisomers (*9*, *10*), we investigated whether these isomers might also differ in their ability to cross the blood-brain barrier.

Following preparative isolation of the diastereoisomers of BH_4 (reduced in this laboratory and composed of a 60:40 mixture of *l* to *d*) (*9*), 0.08 μmole per gram of body weight of either isomer was injected intraperitoneally, and the BH_4 content of the brain was determined (Table 1). Although the natural *l* isomer was

Table 1. Entry of peripherally administered tetrahydrobiopterin and its diastereoisomers into rat brain. Male Sprague-Dawley rats were injected intraperitoneally with *dl*-tetrahydrobiopterin (60 percent *l* isomer) or the isolated diastereoisomers, dissolved in 1 percent ascorbic acid, *p*H 7.0. Control animals received ascorbic acid alone. Values of biopterin accumulated are expressed as means ± standard deviation (S.D.) and represent accumulations above control levels (0.486 ± 0.031 nmole/g).

Treatment	Dose (μmole/g)	*N*	Biopterin accumulated (nmole/g)
dl-Tetrahydrobiopterin	0.10	12	0.466 ± 0.028
l-Tetrahydrobiopterin	0.08	6	0.412 ± 0.064
d-Tetrahydrobiopterin	0.08	6	0.258 ± 0.043

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