References and Notes

1. V. Buonassisi, G. Sato, A. I. Cohen, *Proc. Nat. Acad. Sci. U.S.* **48**, 1184 (1962); B. P. Schimmer, *J. Cell Physiol.* **74**, 115 (1969).
2. D. K. Granner, L. R. Chase, G. D. Aurbach, G. M. Tompkins, *Science* **162**, 1018 (1968).
3. H. Eagle, *ibid.* **130**, 432 (1959).
4. H. J. Strecker and E. E. Eliasson, *J. Biol. Chem.* **241**, 5750 (1966).
5. G. J. Todaro and H. Green, *J. Cell Biol.* **17**, 299 (1963).
6. R. W. Butcher and E. W. Sutherland, *J. Biol. Chem.* **237**, 1244 (1962).
7. M. H. Makman, in preparation.
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
9. M. H. Makman and E. W. Sutherland, *Endocrinology* **75**, 127 (1964).
10. R. J. Boucek and T. R. Alvarez, *Science* **167**, 898 (1970).
11. M. W. Bitensky, V. Russell, W. Robertson, *Biochem. Biophys. Res. Commun.* **31**, 706 (1968).
12. F. Murad, Y. M. Chi, T. W. Rall, E. W. Sutherland, *J. Biol. Chem.* **237**, 1233 (1962).
13. Under the assay conditions described in this report and with the adenyl cyclase preparations used, during the 20-minute assay incubation period ATP breakdown was approximately 35 percent in the absence of NaF, and about 20 percent in the presence of NaF. We have also carried out additional assays modified to include an ATP regenerating system (phosphoenolpyruvate plus pyruvate kinase) or a threefold increase in ATP concentration, or both. These modifications resulted in minimum breakdown of ATP and in elevations of the amount of cyclic AMP produced over that obtained in the assay system routinely used by about 25 percent for both control and catecholamine incubations and by about 10 percent for incubations with NaF.
14. W. L. Ryan and M. L. Heidrick, *Science* **162**, 1484 (1968); M. L. Heidrick and W. L. Ryan, *Cancer Res.* **30**, 376 (1970).
15. H. D. Brown, S. K. Chattopadhyay, H. P. Morris, S. N. Pennington, *Cancer Res.* **30**, 123 (1970).
16. G. Sato, G. Augusti-Tocco, M. Posner, P. Kelly, *Recent Progr. Hormone Res.* **26**, 539 (1970).
17. Supported by NIH grant AM-09213 and career development award 5-K3-AM-25,889. I thank Dr. H. J. Strecker and Miss U. Hammar for advice and assistance in studies with Chang and HeLa cells, Dr. G. Manner for invaluable help in studies with 3T6 and human fibroblast cultures, Dr. J. Warner for HeLa cells, Dr. E. Levine for 3T3 and human fibroblasts, and Mrs. C. Schonfeld and Mrs. D. Daddino for technical assistance.

21 September 1970; revised 20 October 1970 ■

Visual Adaptation in Monkey Cones: Recordings of Late Receptor Potentials

Abstract. *The retinal cones of cynomolgus macaque monkeys show marked amounts of adaptation when the receptor potential is elicited by brief incremental stimuli presented against steady backgrounds of increasing intensity. The results can be accounted for by mechanisms of response compression, modified by the effects of photopigment bleaching, which together set the gain of the system at each background level, while also making the response nearly linear over a significant range of intensities above and below that of each adapting stimulus.*

Discriminations of light intensity can be made from starlight to sunlight, over an intensity range of ten billion to one. The term adaptation describes the ability of the eye to adjust its sensitivity as conditions of external illumination vary over this enormous range.

Some of the mechanisms of visual adaptation are well understood. There is a shift from highly sensitive scotopic (rod) vision to less sensitive photopic (cone) vision that begins about 10,000 times above the rod absolute threshold. Rod signals become saturated at intensities at which photopic vision functions and make no further contribution to intensity discrimination. The pupillary reflex tends to keep retinal illumination constant as more light strikes the eye, but it does not succeed in doing so, being sufficient to account for only a very small fraction of the total photopic adaptation that occurs (*1*).

Adaptation that takes place for fixed pupil size, within the remaining millionfold range of photopic vision, has proved particularly difficult to explain. An early idea was that the bleaching of photopigments was the only additional adaptive mechanism (*2*). According to the reflection densitometry of Rushton and Henry (*3*), bleaching by steady lights reaches about 10 percent at 3.4 log photopic trolands, rises to about 90 percent at 5.3 log trolands, and thus definitely occurs within the normal range of outdoor photopic vision. The primary effect of bleaching is simply to decrease system sensitivity in proportion to the fraction of pigment bleached. For example, if half the photopigment were bleached away, then twice as much light would be required to elicit the same response as before bleaching.

This mechanism is not nearly sufficient, however, to explain photopic adaptation in its entirety, because very large changes in sensitivity are associated with trivial percentages of bleaching (*4*). Thus additional adaptive mechanisms must exist. Some recent theories have proposed that there may be a form of automatic gain control in the retina, with an inhibitory feedback signal being used to control sensitivity (*5*). Although difficult to imagine within receptors, such a system would be plausible if adaptation took place mainly downstream, for example, in the layer of the retina where the bipolar cells are located, as proposed by Dowling (*6*). But this type of proposal is hard to accept as defining the only site of neural adaptation, because it would leave the receptors with an enormous range of intensities to handle, and an explanation of how they do this would still be required. Hence, it seemed likely to us that important adaptive mechanisms, in addition to bleaching, exist within the receptors.

We find that substantial adaptation does occur in photoreceptors and that our results are reasonably well fit by a simple model. The basic mechanism of adaptation in the receptor is the response compression which results from a nonlinear relationship between intensity of stimulation and amplitude of the receptor potential. This is modified in important ways by the effects of bleaching in the case of cones, but probably not for rods.

Using the technique of K. T. Brown (*7*), we have isolated and recorded the late receptor potential (late RP) from cynomolgus macaque monkeys, which with respect to vision are near relatives to man. There is good evidence that the late RP is the signal that is transmitted along the receptor, playing a crucial role in the activation of second-order cells (*8*). In our work this potential was recorded extracellularly with a microelectrode whose tip was located in the outer-segment layer of the receptors by methods described previously; the tip was directed toward the macular region where cones predominate. The position of the electrode tip in the macula was confirmed by direct observation. Also, we have accumulated more than 40 spectral sensitivity functions (*9*) which indicate that under the conditions of stimulation in this work the responses come only from the three types of cone receptors that have been identified in the primate retina. The many cones in the immediate region of the electrode generate tiny potentials that summate to produce a recordable response, whose size varies from about 2000 μv at high intensities down to 10 μv or less at the lowest intensities that we have used, where the signal is lost in noise despite the response averaging procedures that we used throughout.

The basic experiment consisted of presenting steady light fields to the eye, allowing sufficient time for a stable response voltage to be reached. Incremental flashes 150 msec long were then added to the same area of the retina. When presented against any given steady background, the size of the response to an incremental flash was found to grow with incremental intensity, whereas the response to an incremental flash of fixed intensity decreased markedly as the background intensity was raised to higher values. As expected from this, the incremental intensity required to generate a criterion response increased with background intensity, producing the typical curve of log threshold as a function of log intensity shown in Fig. 1A. Psychophysical data are also presented for a human observer using the same optical stimulator. The similarity of the two curves indicates that a major part of the adaptive machinery is in the cones themselves (*10*).

In other experiments we measured the amplitude of the response to incremental flashes against various backgrounds, using a wide range of intensities of each (Fig. 1B).

In Figs. 1B and 2, the curve for R_{t_0}, an incremental response to a test flash in the special case of zero background, is described by the nonlinear equation

$$R_{t_0} = \frac{I_t^{\,n}}{I_t^{\,n} + K_r} \qquad (1)$$

where I_t is stimulus intensity in trolands and K_r is the number of trolands for half-saturation of the receptor potential. When I_t is sufficiently low that $I_t^{\,n} \ll K_r$, which implies that the saturation level of the receptor is very distant, it may be seen that Eq. 1 is a simple power law with exponent n. In many experiments we find n to be near 0.70. This means that, even at very low intensities, the increment of the receptor potential to the absorption of a quantum is less, the more quanta have been absorbed previously within a certain space-time integral. Regardless of the value of n, the response R_{t_0} reaches half its maximum value when I equals K_r. Most of our data are well fit when values of K_r equal to 831 trolands (*11*) and of n equal to 0.73 are chosen.

We propose that the fundamental reason why the presence of a background reduces the response to an incremental flash is that it produces a steady receptor response to which the incremental response must add. The nonlinearity expressed by Eq. 1, reflecting a power-law response and the effects of saturating the receptor potential, produces a response compression which progressively reduces the effectiveness of a given incremental flash as the responses to the adapting field become larger (*12*). If there is no bleaching, the response to the adapting field plus the test can be expressed as

$$R_{a+t} = \frac{(I_a + I_t)^n}{(I_a + I_t)^n + K_r} \qquad (2)$$

Equation 2 implies that, if we measure the total response from a stimulus baseline of zero to the peak of an incremental response that is superimposed upon the response to the background, then the full response magnitude should be predicted by the linear sum of the intensities of the background and test stimuli, no matter how distributed between the two components of the stimulus. We have tested and confirmed this prediction by using transient background flashes too brief to permit significant bleaching.

Equation 1 also applies to an adapting field that does not bleach, so that R_a equals R_{t_0} and we may write

$$R_a = \frac{I_a^{\,n}}{I_a^{\,n} + K_r} \qquad (3)$$

To predict the effect of such an adapting field upon the response to the test superposed upon it, subtract Eq. 3 from Eq. 2:

$$R_t = R_{a+t} - R_a \qquad (4)$$

We propose that Eq. 4 is sufficient to describe adaptation of the receptors, but it cannot be solved in the general case unless R_a is known.

To calculate R_a, we make use of the kinetics of cone bleaching as determined by Rushton, from which the fraction x of pigment bleached at steady state can be calculated as

$$x = \frac{I}{I + K_b} \qquad (5)$$

where K_b is the number of trolands required for 50 percent of the pigment to be bleached. The value of K_b

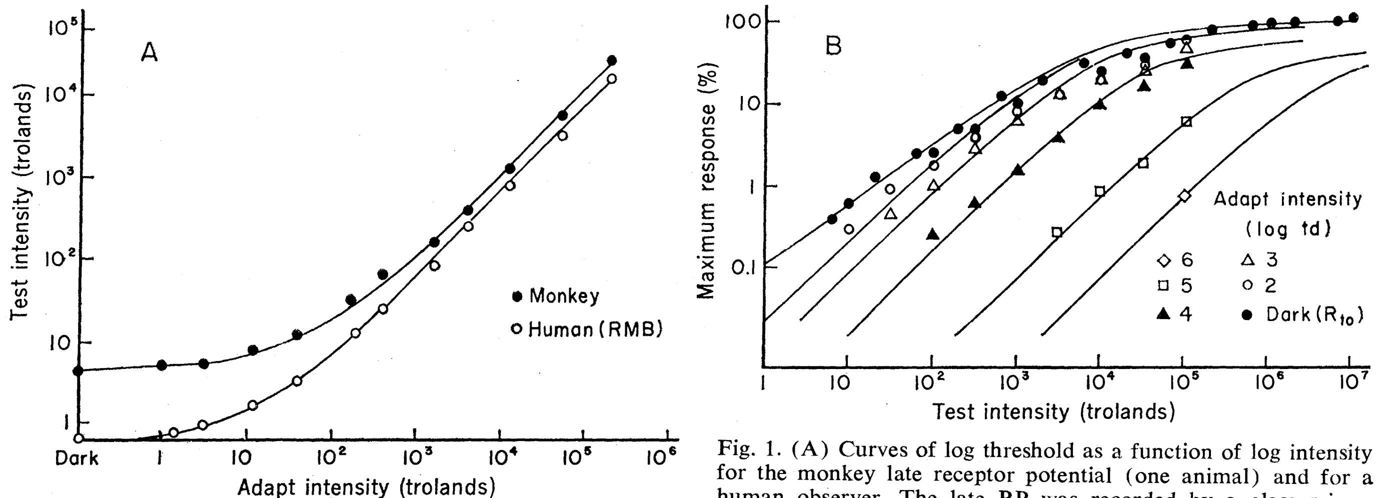

Fig. 1. (A) Curves of log threshold as a function of log intensity for the monkey late receptor potential (one animal) and for a human observer. The late RP was recorded by a glass micropipette whose tip was in the fovea at the depth of the cone outer segments. Electrical responses were averaged, and amplitudes were measured at the termination of the stimulus. A 10 μv criterion was taken as threshold. The curve for the human was obtained with the same optical stimulator and a method of adjustment, but with the test flash confined to the fovea. In both cases the subject was adapted to a steady intensity of yellow light, fixated centrally for 5 minutes, and tested with 150-msec flashes of yellow (580 nm) light. (B) Intensity series of the monkey late RP at various steady adapting intensities and for the dark-adapted case. The adapting and test fields were both the same size (1.1 mm circular spots) and were centered upon the fovea. The adapting intensities are given in log photopic trolands. The curves drawn through the data points are theoretical and are described in the text. The curves drawn through the data for 5 and 6 log trolands (*td*) of adaptation are the theoretical curves for 5.3 and 6.3 log photopic trolands, respectively. The data are the mean values from six animals; they have been normalized as the percentage of the dark-adapted response at 6 log trolands (usually about 2000 μv).

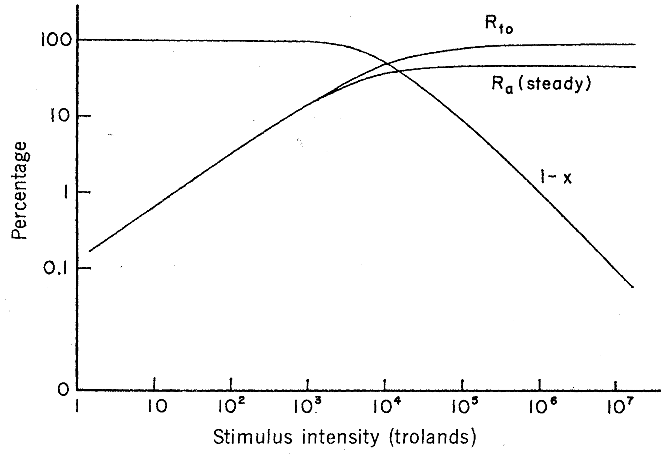

Fig. 2. Theoretical curves. R_{to} is the basic response curve of dark-adapted cones, whereas R_a is the cone response to steady illumination, normalized to 100 percent as the maximum response. The quantity $(1 - x)$ is the fraction of photopigment remaining unbleached (shown as a percentage on the graph) at any steady illumination of given intensity. All are scaled logarithmically. Note that R_{to} is confirmed in Fig. 1B with 150-msec flashes, and R_a has also been confirmed experimentally.

for humans is given by Rushton as 20,000. Since the monkey has a smaller eye, a given troland value will be more effective than in the human. In our work we set K_b equal to 10,000.

The effectiveness of a light stimulus can be expressed in terms of an equivalent intensity, I', such that

$$I' = I(1 - x) \qquad (6)$$

Equation 6 describes the basic effect of bleaching as outlined at the outset—bleaching that affects the test stimulus as well as the adapting stimulus, neither of which will be as effective upon the bleached eye as upon the unbleached one. If we substitute I'_a for I_a in Eq. 3 and I'_t for I_t in Eq. 1 and then substitute into Eq. 4, we get:

$$R_t = \frac{(I'_a + I'_t)^n}{(I'_a + I'_t)^n + K_r} - \frac{I'^{\,n}_a}{I'^{\,n}_a + K_r} \qquad (7)$$

We used Eq. 7 to calculate the theoretical curves of Fig. 1B. The values used for n and K_r (0.73 and 831, respectively) were based upon experiments in which we recorded dark-adapted series of response amplitude as a function of stimulus intensity. The last term of Eq. 7 is the theoretical response to a steady adapting field. We estimated R_a directly using brief dark intervals following various steady state levels of adaptation and found that the size of the negative response agreed with the calculated results (*13*).

In Fig. 2 the various functions described by the equations are shown graphically. Note that R_a, the response to a steady adapting field, saturates at half the maximum response amplitude for a short flash. This means that, no matter how intense the adapting field, the receptor response R_a cannot be driven beyond a certain intermediate voltage. Thus when the eye is fully adapted to the prevailing intensity, bleaching precludes the receptor response from saturating, so that the receptor always operates on a sensitive portion of its characteristic response function.

Our interpretation seems to eliminate entirely the requirement for an "eigengrau"—the receptor noise frequently invoked to interpret failure of Weber's law at low intensities (*14*). By our scheme, dark-adapted thresholds for cone receptor potentials simply depend upon the intensity required to drive the response to a criterion voltage. The initial effects of light adaptation, at low intensities before bleaching enters, result from response compression. The adapting stimulus sets receptor sensitivity to a new range by causing the incremental response to rise from progressively lower-sloped portions of the characteristic response curve R_{to}. At high adapting intensities, bleaching enters and limits the amplitude of the signal R_a. At these high intensities of adaptation the increment response always rises from the same portion of the response curve, so the receptors can respond efficiently at indefinitely high adapting intensities. As suggested by Alpern *et al.* (*15*) this is probably why the psychophysical curve of threshold as a function of intensity also does not saturate for cone vision. Rods are not similarly protected because bleaching is quantitatively small at intensities which saturate the rod receptor potential.

An important prediction of our theory is that the amplitude of the receptor signal is nearly linearly proportional to stimulus intensity for a considerable range on either side of the voltage set by the adapting field. This prediction has been verified for increments in our work, and is also in agreement with observations of Rodieck and Ford (*16*) in the cat. However, we cannot confirm the conclusion reached by Alpern *et al.*, based on psychophysical evidence (*15*), that receptor signals are a linear function of intensity over a range of several log units (*17*). We find that receptor signals are linear (or nearly so), but only if the eye is first adapted to each intensity before the test for linearity is made.

Within the range in which it occurs, the nearly linear receptor response, as appropriately attenuated at each higher adapting level, identifies a basic mechanism of brightness constancy at the earliest stage of the visual system. Linearity also has important implications for theories of color vision, contrast modulation, areal summation, or any others that assume linearity of physiological response over a certain range of intensities above and below that of the adapting stimulus.

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References and Notes

1. The facts cited in this paragraph have a long history and are generally accepted. Textbooks providing more details and citations of primary references include: C. H. Graham, Ed., *Vision and Visual Perception* (Wiley, New York, 1965); Y. LeGrand, *Light, Colour, and Vision* (Chapman and Hall, London, ed. 2, 1968); H. Davson, Ed., *The Eye* (Academic Press, New York, 1962).
2. S. Hecht, *Physiol. Rev.* **17**, 239 (1937).
3. W. A. H. Rushton and G. H. Henry, *Vision Res.* **8**, 617 (1968).
4. W. S. Stiles, *An. Real Soc. Espan. Fis. Quim. Ser. B Quim.* **57**, 149 (1961); G. Wald, *Science* **119**, 887 (1964).
5. R. M. Boynton, *J. Opt. Soc. Amer.* **53**, 165 (1963); W. A. H. Rushton, *Proc. Roy. Soc. London* **162B**, 20 (1965).
6. J. E. Dowling, *Science* **155**, 273 (1967).
7. K. T. Brown, *J. Opt. Soc. Amer.* **54**, 101 (1964); K. T. Brown, K. Watanabe, M. Murakami, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 457 (1965).
8. K. T. Brown and M. Murakami, *Vision Res.* **7**, 179 (1967); K. T. Brown, *ibid.* **8**, 633 (1968).
9. R. M. Boynton and D. N. Whitten, in preparation.
10. The rather close agreement between our data on humans and monkeys (Fig. 1A) should not be taken to suggest that important adaptive mechanisms do not also exist beyond the receptor level. The shape of the curve for humans depends upon retinal area and

stimulus duration; with small, brief flashes this curve would have started higher and would have a lower slope over most of its range. Although we have not yet tested for it, it is possible that the curve for the monkey also depends upon such parameters, but probably for very different reasons.

11. The constant K_r is equal to $(10,000)^{.70}$. The value of 10,000 is also the bleaching constant of Eq. 5. We are uncertain whether this agreement is fortuitous, or of theoretical significance.
12. There are scattered references to the concept of response compression [see, for example, R. M. Glantz, *Vision Res.* **8**, 1407 (1968); G. Werner and V. B. Mountcastle, *J. Neurophysiol.* **28**, 359 (1965); K. I. Naka and W. A. H. Rushton, *J. Physiol.* **185**, 587 (1966)]. Naka and Rushton discussed response compression but dismissed it as not fitting their data from fish S-potentials. Glantz used the concept implicitly to describe light adaptation in crayfish photoreceptors. Werner and Mountcastle used it to construct Weber functions for vertebrate mechanoreceptors, successfully mimicking human psychophysical results. The latter two studies both found a power law relationship between stimulus and response, and both used an equal response increment as the threshold criterion, just as we have done.
13. Consider the case where I_a equals 6 log trolands, which is sufficient to produce an essentially full bleach at steady state. In this case, if the light is delivered suddenly to the dark-adapted eye and left on, the late RP should rise quickly to its full value and then gradually settle down to half that value as bleaching progresses and the probability of quantum absorption becomes less. Although we observed this qualitatively, we could not measure it this way because the time course is too slow relative to the drift in the recording situation. One can, however, make the prediction that, if the steady-state adapting field at 6 log trolands is momentarily extinguished, a "negative response" should be produced that has just half of the response to a brief positive flash delivered to the previously dark-adapted eye. At the other extreme, where I_a is too weak to produce significant bleaching, such positive and negative responses should be of the same size. These predictions, and others applying to intermediate conditions of intensity, were experimentally tested and confirmed within experimental error.
14. See, for example (*9*) and H. B. Barlow, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 539 (1965).
15. M. Alpern, W. A. H. Rushton, S. Torii, *J. Physiol.* **207**, 463 (1970).
16. R. W. Rodieck and R. W. Ford, *Vision Res.* **9**, 1 (1969).
17. Contrary to the assertion of Alpern *et al.* that electrophysiological signals from receptors and S-potentials are linear and fit by the expression V equals $I/I+K$, we find that I in the expression must be raised to an exponent less than 1.0. This is also the case with cat S-potentials [R. Steinberg, *Vision Res.* **9**, 1331 (1969)], and from some of Steinberg's more recent work (personal communication). The confusion arises from the close similarity of the curves V equals $I/I+K$ and V equals I^n/I^n+K^n when plotted (as is the convention) on semilog coordinates.
18. We thank K. T. Brown and R. H. Steinberg for helpful discussions, and Mrs. P. Lacy for technical assistance. This work was done while R.M.B. was on leave from the University of Rochester (to which he has returned); he was supported by NIH special fellowship NB-00693-02. The work was supported also by NIH grants EY-00187 and EY-00468.

5 August 1970

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Superior Colliculus: Single Unit Responses to Stimulation of Visual Cortex in the Cat

Abstract. *Electrical stimulation of area 18 of the cat visual cortex produces intense excitation of neurons in the superior colliculus. Excitation is followed by a period of decreased collicular responsiveness to light stimulation. These effects are seen in both directionally selective and nonselective units. The corticocollicular projection is retinotopically organized.*

Anatomical studies have repeatedly demonstrated substantial projections from the visual cortex to the superior colliculus in several species, including the cat (*1*). In recent years physiological and behavioral experiments have indicated that these connections mediate important functional relations between the cortical and tectal components of the visual system (*2–7*). Evidence from these studies has implicated both excitatory and inhibitory mechanisms in the corticocollicular influence. The experiments reported here show that electrical stimulation of the visual cortex produces excitation followed by functional inhibition of collicular neurons.

Action potentials of single neurons in the superior colliculus were recorded with tungsten microelectrodes in midpontine pretrigeminal cats prepared under ether anesthesia. The animals viewed a tangent screen at a distance of 1 m, and their eyes were corrected for this distance (*8*). After completion of surgery, the animals were paralyzed with Flaxedil and given artificial respiration. Expired CO_2 and femoral arterial pressure were continuously monitored, and body temperature was maintained at 37° to 38°C. The ipsilateral occipital cortex was covered with warm mineral oil and stimulated with a movable silver ball electrode, 0.5 mm in diameter. The other electrode pole was placed in surrounding muscle or grounded to the stereotaxic instrument. Bipolar stimulation of the visual cortex was used in some experiments. A Hewlett-Packard current probe monitored stimulus current. When a collicular unit was isolated, its receptive field was characterized by moving patterns on the tangent screen, which was diffusely illuminated at a constant photopic level. The unit's response to cortical stimulation was determined, and, finally, interactions between effects of light and cortical shock were investigated. Data were recorded on film and on magnetic tape for subsequent analysis. Recording sites were determined histologically from small electrolytic lesions placed in the electrode tracks.

Single electrical pulses to the visual cortex evoked a short latency discharge in superior collicular neurons (Fig. 1). As we gained skill in placing the cortical electrode (see below), this response was observed in all collicular units investigated. The typical response to a 0.5-msec current pulse to the ipsilateral visual cortex was a burst of spikes, often of very high frequency, which occurred during a negative evoked wave (Fig. 1). Latency to the first spike was variable at suprathreshold current intensities, which indicates orthodromic activation. Minimum latencies ranged from 1.2 to 9.0 msec with a mean of 3.17 ± 1.96 msec (standard deviation) in 26 units (*9*). The values are comparable to the 2.0- to 2.5-msec latencies of evoked potentials in the superior colliculus after cortical shock (*6*) and spontaneous strychnine spikes (*7*). Hayashi has described antidromic invasion of visual cortical units, after stimulation of the superior colliculus, with a mean latency of 2.8 ± 1.6 msec (standard deviation) in 60 cells (range of 0.9 to 9.0 msec) (*10*). The projecting fibers studied by Hayashi clearly provide a possible pathway for the excitatory effects that we have observed.

After the phase of synaptic excitation, the superior collicular neurons exhibited a period of depressed responsiveness which lasted for 50 to 100 msec, depending on cortical shock intensity. Figure 2 illustrates a control off response, which is markedly reduced when preceded by a cortical shock (lower two traces). In this case the cortical shock elicited a burst of spikes from the superior collicular neuron. It was commonly observed, however, that the cortical stimulus did not have to excite a unit in order to produce detectable inhibition. On the other hand, careful adjustment of cortical and visual stimulus parameters revealed the inhibitory phenomenon in the majority of units driven from the visual cortex.

All units studied in the superior colliculus were classified as directionally selective or directionally nonselec-