Reports

A Mechanism of Light Adaptation

Abstract. In the isolated retina of the bullfrog (*Rana catesbiana*) illumination of one part of a ganglion cell's receptive field increased the light threshold (for response by that cell) not only in the illuminated part but also in the unilluminated parts of the field. Scattered light is insufficient to account for the effect. Apparently it depends on changes in the efficiency of excitation transmission along the neural pathways from photoreceptors to ganglion cell.

Visual thresholds rise on exposure to increased illumination (light adaptation) and fall on exposure to decreased illumination (dark adaptation). Five hypotheses have been advanced in explanation. The first, that these processes are due entirely to bleaching and regeneration, respectively, of rhodopsin, has been shown to be untrue (*1*), except possibly at high light intensities. The second, that changes occur in the spatial summation of light stimuli incident on the retina, has been found to account for only a 20-fold threshold change out of a 3000-fold total in the human being (*2*). The third, assuming similar changes in temporal summation, has not been adequately tested, but it is unlikely that it will account for any greater threshold change than the second. The fourth is that a rod consists of many compartments, each inactivated by the photolysis of any one of the molecules of rhodopsin in it—the rod's output activity being proportional to the number of photons absorbed in intact compartments (*3*). This would result in the threshold's being related, though not in inverse proportion, to the amount of rhodopsin present. The fifth is that the efficiency with which excitation is transmitted from the receptors to the optic nerve is changed during light or dark adaptation. The last two hypotheses were tested over the lower 600-fold threshold change by the experiments described below.

In each experiment the excised retina of a dark-adapted bullfrog (*Rana catesbiana*) was placed receptor side up in a moist chamber and supplied with a moistened mixture of 95 percent oxygen and 5 percent carbon dioxide. A microelectrode pierced down through the retina to contact the body or axon of a single ganglion cell. Its action potentials were amplified and presented on an oscilloscope and a loudspeaker. In contact with the underside of the thin glass cover slip forming the chamber's bottom was a metal diaphragm having a tiny opening. The chamber and electrode holder were mounted on a stage that was movable with respect to the diaphragm, and whose position could be determined within 5 μ by micrometer screws. Light from a tungsten filament lamp, of an intensity determined by a neutral density wedge, passed through an electromagnetic shutter, a system of lenses and mirrors, and the diaphragm, entering the retina in the natural direction from the ganglion cell side and forming an oval light spot about 100 by 160 μ at the receptors.

Threshold was tested with ½-second light flashes at 5-second intervals. Threshold was taken as that intensity which elicited three responses (discharge of action potentials) from the ganglion cell in four successive flashes. These responses are known to be rod-dominated (*4*). The retinal region (receptive field) in which illumination elicited a response was located. Micrometer screws at the left and right of the stage were set so that movement of the stage into contact with one or the other would direct the light spot onto either of two selected areas in the receptive field. One area was illuminated continuously for several minutes. Then the thresholds were measured alternately at the (previously) illuminated and the unilluminated areas to determine how much each had changed, and to follow their dark adaptation (*5*).

The unilluminated area, as well as the illuminated one, showed a rise in threshold. To determine whether this was caused by light scattered from the illuminated to the unilluminated area, use was made of measurements taken previously on the same apparatus of the light-scattering properties of the frog retina (*6*). An upper bound was computed for the average intensity of light scattered from the illuminated area to the rods of the unilluminated area. Direct illumination of the latter with the average or a greater intensity produced a threshold rise which in nine out of ten areas so tested (in three different retinas) was less than the rise to be explained. In the tenth it was slightly greater. Since these upper bounds exceed the actual light scatter, these experiments showed that little, if any, of the threshold rise in the unilluminated area was caused by scattered light.

In the experiment illustrated in Fig. 1, area 1 was the most sensitive region of the receptive field, and area 2 was more peripheral by 350 μ, with 100 times higher threshold. Area 1 was illuminated for 10 minutes with 315,000 times its threshold intensity. This caused a greater rise in area 2's threshold than did the same illumination directed onto area 2. The same was true in three other

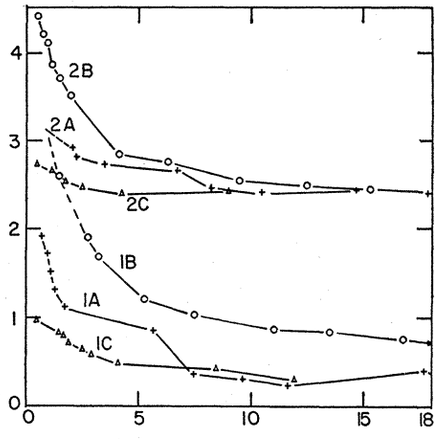

Fig. 1. Dark adaptation of illuminated and unilluminated areas in the receptive field of a frog's retinal ganglion cell after 10 minutes of illumination of one of the areas. Ordinate: common logarithm of the relative light intensity. Abscissa: minutes in the dark. Curves *1A* and *2A*, thresholds of areas 1 and 2, respectively, after illumination of area 2. Curves *1B* and *2B*, thresholds of areas 1 and 2, respectively, after illumination of area 1. Both illuminations were made with light of 5.5 logarithmic units relative intensity. Curve *1C*, threshold of area 1 after illumination of area 1. Curve *2C*, threshold of area 2 after illumination of area 2. Both illuminations were made with light of 3.5 logarithmic units relative intensity. For the first two illuminations the computed upper bound of light scatter from one area to the other was 2.3 logarithmic units relative intensity. Note that *1A* exceeds *1C*, and that *2B* exceeds both *2C* and *2A*.

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

central-peripheral pairs of areas so tested.

In another experiment, in a single receptive field the effect of illumination at the center was compared with illumination of three areas peripheral by various distances. The threshold rises evoked centrally by peripheral stimulation, and vice versa, did not decrease rapidly with increased separation (as would be expected if caused by scattered light), but remained roughly the same over the whole range of separations (300–810 μ).

For a fixed duration of light adaptation, it was found that $I_{2t}/I_{1t} \approx kI_a/I_{1i}$; where I_{2t} is the post-adaptation threshold (measured after 1 minute in the dark) and I_{1t} is the pre-adaptation threshold, both for the tested area; k is a constant; I_a is the adapting intensity; and I_{1i} is the pre-adaptation threshold of the illuminated area. If illumination and testing are done on the same area, I_{1t} and I_{1i} become the same. This relation held no matter which was the illuminated and which the tested area in the given receptive field. It held, and with the same constant of proportionality, for four receptive fields, all "on-off" type, each in a different retina, in which a total of 31 adaptations were measured.

These findings indicate that the fraction of excitation reaching the ganglion cell from illuminated receptors anywhere in the receptive field is reduced in proportion to the amount of activity just previously sent toward the ganglion cell from illuminated receptors in the same or a different region of the receptive field. The resultant threshold rise found on testing any group of receptors is not dependent on their previous exposure to light (and any consequent changes in their visual pigments), but apparently is a change in the efficiency of excitation transmission along the neural pathways to the ganglion cell (*7*).

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References and Notes

1. For a review of this question, see G. S. Brindley, *Physiology of the Retina and the Visual Pathway* (Williams and Wilkins, Baltimore, 1960), pp. 180–185.
2. G. B. Arden and R. A. Weale, *J. Physiol.* **125**, 417 (1954).
3. G. Wald, *Science* **119**, 887 (1954).
4. K. O. Donner and R. Granit, *Acta Physiol. Scand.* **17**, 161 (1949); R. Granit, *Sensory Mechanisms of the Retina* (Oxford Univ. Press, London, 1947), p. 269.
5. This experimental approach is based on one suggested by W. A. H. Rushton.
6. L. E. Lipetz, *Abstracts of Fourth Annual Meeting of the Biophysical Society* (1960), p. 25.
7. This work was supported by a research grant, B-1408, from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service, through a contract with the Ohio State University Research Foundation.

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Plaque Reduction, a Sensitive Test for Eastern Encephalitis Antibody

Abstract. Serologic surveys of vertebrates to determine rates of eastern encephalitis infection were made to discover the most likely disseminators of virus in nature. Of the techniques available, neutralization is the most specific, and antibody is known to persist for many years. This communication reports a fivefold increase in sensitivity of neutralizing-antibody detection by the application of a plaque-reducing technique.

Itoh and Melnick (*1*) were able to detect antibody to ECHO type 4 virus by plaque reduction when standard tube techniques failed to reveal it. They defined the titer of a serum as the highest dilution which reduced the number of plaque-forming units by 80 percent. The use of this method for detecting eastern encephalitis antibody was suggested by Henderson and Taylor (*2*).

Adequate numbers of sera, in adequate amounts, were not available for testing from natural infections; only 11 human sera, collected 1½ to 22 years after infection with the virus, were available. To induce infection, five wild rabbits, five crows, two blue jays, four pigeons, five chickens, five frogs, and four squirrels were inoculated subcutaneously with eastern encephalitis virus. All animals which became viremic produced plaque-reducing neutralizing antibody and maintained it throughout the 9 months of serial bleedings (group 1). The animals in which viremia was not demonstrated failed, with one exception, to produce antibody, and blood samples from these animals, together with samples from pre-inoculation bleedings, comprised the controls (group 2). Blood samples from these various sources plus 91 samples from field specimens of birds and mammals (group 3) made a total of 229 sera examined in parallel by two methods.

Chick embryo tissue cultures were prepared, as described previously (*3*). The planting medium, Hanks-Eagle-pyruvate 1-percent fresh egg albumin (HEPA), was supplemented with 1-percent horse serum to obtain a continuous sheet of cells in 60-mm plastic petri dishes. After 48 hours of incubation the medium in tube cultures was replaced with "change medium" HEPA, buffered with tris hydroxymethyl amino methane (*p*H 7.8) (no bicarbonate and no serum). The planting medium was removed from the plates just before inoculation.

Sera were diluted 1:5, inactivated at 56°C for 30 minutes, and incubated for 1 hour at room temperature with an equal volume of eastern encephalitis virus at a dilution calculated to contain 200 $TCID_{50}$ per 0.1 ml. Two plates and two tube cultures were inoculated with 0.1 ml of each serum-virus mixture. After 1 hour of adsorption at 36°C, the plates were overlaid, without washing, with 4 to 5 ml of "change medium" containing 1 to 1.5 percent of agar. After 48 to 72 hours' incubation at 36°C, the plates were stained with neutral red (1:10,000) in saline.

The plaque-forming units of several control plates were averaged in each of nine experiments; the average number of units ranged from 25 to 80. In tube cultures, hemagglutination of goose red blood cells by supernatant fluids (*4*) served to differentiate specific viral cytopathogenic effects from other tissue-destroying factors, such as toxic sera.

Table 1. Detection of eastern encephalitis neutralizing antibody: results of tests by two methods, in parallel, of serum-virus mixtures. Group 1, sera (N = 11) from human beings who had recovered from eastern encephalitis, and sera (N = 97) from inoculated animals after viremia had been demonstrated. Group 2, sera from animals before inoculation, and after inoculation when no viremia had been demonstrated. Group 3, sera from field specimens (birds and mammals).

Plaque-reduction neutralization*			Tube-culture neutralization†			
Positive	Equivocal	Negative	Positive	Equivocal	Negative	Total
			Group 1, infected			
82			18	11	53	82
	16		0	1	15	16
		10	0	0	10	10
			Group 2, controls			
3‡			0	0	3	3
	0		0	0	0	0
		27	0	0	27	27
			Group 3, unknowns			
7			0	2	5	7
	2		0	0	2	2
		82	0	0	82	82
			Totals			
92	18	119	18	14	197	229

* Categories for reduction of plaque count of the serum-virus mixtures are as follows: (positive) 0 to 20 percent of the average count of plaque-forming units of virus controls; (equivocal) 21 to 30 percent; (negative) 31 percent or more. † (Positive) 2:2 cultures, no virus growth; (equivocal) 1:2 cultures, no virus growth; (negative) 0:2 cultures, no virus growth. ‡ Three blood samples from a single inoculated wild rabbit; presumably viremia was missed.