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13. PR_{50} means plaque reduction assay where 1 PR_{50} unit (VSV) represents the concentration of interferon that will reduce the plaque titer of VSV by 50 percent in a standard assay system [R. R. Wagner, *Virology* **13**, 323 (1961)]. Mouse interferon purified by zinc acetate (Dr. C. Colby) showed 50,000 PR_{50}-VSV unit/ml assayed on mouse L cells. Interferon or mock interferon prepared from chick embryo cells aged in vitro (*14*) and preparations purified with zinc acetate usually contained 10,000 PR_{50}-VSV unit/ml assayed on 3-day monolayers of chick embryo cells. Preparations obtained after perchloric acid precipitation and neutralization with KOH produced comparable results.
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16. By the usual convention the virion (parental or genome) strand of RNA is termed plus (+). The translating or messenger strand of most RNA viruses is a (+) strand or messenger RNA (mRNA). However, in VSV there is evidence that the translating or message strand has a base composition complementary to virion RNA [F. L. Schaffer, A. J. Hackett, M. E. Soergel, *Biochem. Biophys. Res. Commun.* **31**, 685 (1968)]. If the message strand, rather than the virion strand, on polysomes of infected cells is a common reference point, then almost all viruses contain a (+) strand (mRNA) in the virion. However, by this convention, VSV contains a (−) strand in the virion and the virion polymerase produces a (+) strand or mRNA. As noted by Baltimore *et al.* (*1*) the virion polymerase of VSV is probably a transcriptase rather than a replicase.
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3 May 1971; revised 22 August 1971 ■

Light Adaptation in the Rat Retina: Evidence for Two Receptor Mechanisms

Abstract. *Light adapting the rat retina with transient white flashes too dim to bleach a substantial amount of visual pigment produces a change in electroretinogram spectral sensitivity and an increase in flicker fusion frequency. Increment threshold curves obtained with a long wavelength adapting stimulus and a short wavelength test stimulus show rod saturation.*

Although it is generally agreed that some of the visual receptors in the rat differ from typical rods, experiments on the rat eye are frequently interpreted as if responses reflected the activity of rods alone (*1*, *2*). This report provides new evidence that at higher levels of light intensity a second type of receptor contributes to the rat electroretinogram (ERG). The starting point for these experiments was two aspects of rat vision which differ from rod vision in the human eye.

First, light adaptation produces a spectral sensitivity that differs from the rod rhodopsin spectral sensitivity of the dark-adapted animal in that it is more sensitive in the long wavelength region (*3*, *4*). In man such shifts signify a shift from rods to cones. Second, increment thresholds increase proportionately with increases in background (*5*) over a range of intensities which far exceeds those found for the rods in man, cat, and monkey. The rods in these latter animals saturate under moderate conditions of illumination (*6*–*8*). The shift in spectral sensitivity and the failure to find rod saturation might be interpreted as evidence for a second receptor; however, there are other possible explanations. For example, as Dowling (*2*) has pointed out, at low levels the visual pigment in the rods is likely to act as screening pigment in front of the reflective postretinal tissues. Adapting the retina to levels that bleach a significant amount of visual pigment could remove this screen and produce a shift in spectral sensitivity due to the increased reflectance from postretinal tissues. Another possibility is that the bleaching of visual pigment produces photoproducts (*9*) which filter the incident light.

Not only might bleaching explain spectral sensitivity changes, it could also account for the failure to find rod saturation. As has been shown for human cones by Alpern *et al.* (*10*), pigment bleaching by a steady background

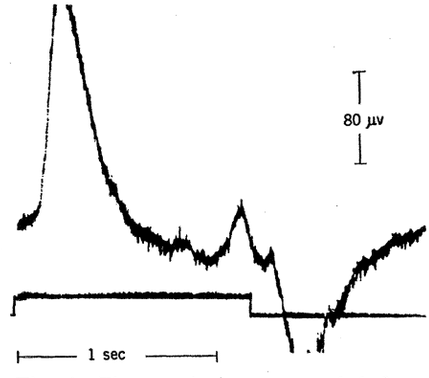

Fig. 1. Electroretinogram recorded from albino rat in response to a test flash which follows 1 second after the onset of a brief adapting stimulus. The lower tracing marks the duration of the adapting stimulus. The onset of the adapting stimulus produced a large *b*-wave response that carried the tracing off the oscilloscope screen. The smaller positive response appearing just before the adapting light turns off is the incremental response to the test stimulus.

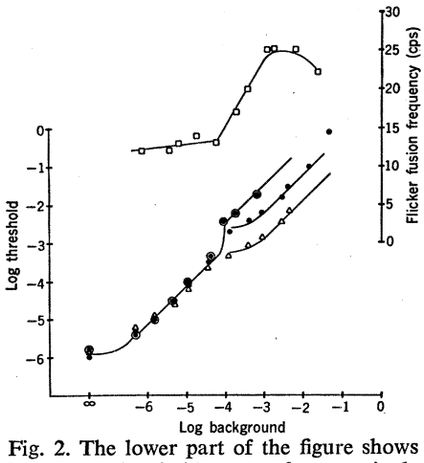

Fig. 2. The lower part of the figure shows increment threshold curves from a single albino rat. Each point represents a determination of the relative energy in the test required to produce a 40-μv incremental response. The blue and red test stimuli used in making threshold determinations were equated on the spectral sensitivity curve of the dark-adapted rat retina. The three sets of measurements are for a blue test on a white background (black dots), a red test on a white background (triangles), and a blue test on a red background (circles containing dots). To equate the intensity of the red and white backgrounds, the measurements made with the red background were shifted horizontally until the two sets of determinations coincided at the lowest levels of adaptation. The squares in the upper part of the figure are determinations, from the same animal, of ERG flicker fusion frequency as a function of mean intensity of a flashed and flickering white field.

can obscure receptor saturation. To demonstrate these effects experimentally, Alpern *et al.* used brief adapting flashes to eliminate bleaching. I have modified their psychophysical technique for use with the rat ERG.

Figure 1 shows a typical albino rat ERG response to a 1.2-second adapting stimulus with a superimposed short test flash. When the background is turned on, the sensitivity of the retina is so quickly reduced that a test stimulus following the onset of the background by 1 second can be used to measure changes in retinal sensitivity. The adapting stimulus is presented for such a brief period that it bleaches no significant visual pigment. It then becomes possible to differentiate the effects produced by bleaching from those resulting from receptor duplicity.

The recording procedures I used were similar to those used by Cone (*11*). Twelve rats (11 albino and one pigmented) weighing between 250 and 350 g were used. The animals were dark adapted for 12 hours or more, then anesthetized with urethan (200 mg/100 g) injected intraperitoneally. Under dim red illumination, the eyelid of one eye of each rat was drawn back with sutures, the pupil was dilated with atropine sulfate (1 percent), and the animal was taped into position on its side. Electroretinograms were recorded between a small cotton wick electrode positioned on the cornea under a third of the Ping Pong ball and a chlorided silver wire placed in a small cut at the side of the nose. The recorded signals were amplified by a Tektronix 122 preamplifier with a time-constant setting of 0.2 second and were displayed on a storage oscilloscope. The light from a 150-watt xenon arc lamp, the background, was optically combined with light from a xenon flash tube, the test, and imaged on the top surface of the Ping Pong ball.

The incremental test intensity required to produce a 40-μv ERG response was determined with flashed backgrounds of increasing intensity. In order to measure both light adaptation and spectral sensitivity, two test lights of different spectral composition were used to generate criterion responses. These were obtained by inserting a Kodak Wratten No. 47 [a short wavelength band-pass filter (blue)] and a Kodak Wratten No. 92 [a long wavelength filter (red)] in front of the test stimulus. Increment thresholds were obtained by adjusting the intensities of these two test stimuli until they both produced criterion responses when combined with the flashed xenon white background.

These measurements showed that with an adapting background too brief to bleach there was a break in the increment threshold curves at a background of about -4 log units (Fig. 2). Light adaptation with backgrounds above -4 log units produced a long wavelength shift in the spectral sensitivity of the rat's ERG. These effects suggested that the increment threshold curve measured against bright backgrounds was determined by a second receptor mechanism, more sensitive to long wavelengths than the mechanism determining low-level thresholds. If this were the case it should have been possible to differentially depress the sensitivity of the second mechanism by using a long wavelength adapting stimulus. This expectation was in fact borne out. Moreover, the red adaptation produced an increment threshold curve which at a particular background deviated from its initial ascent,

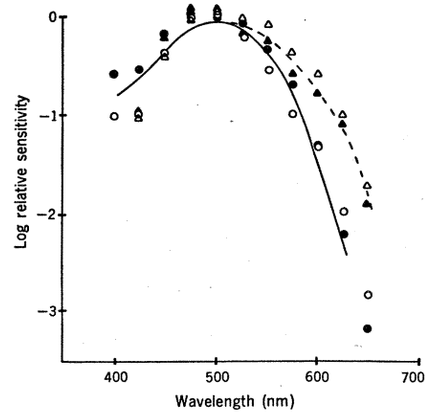

Fig. 3. Spectral sensitivity of the light- and dark-adapted rat retina. Circles (○, ●) indicate the relative energies required to produce a criterion response in the dark-adapted retina. The triangles (△, ▲) are similar determinations after transient light adaptation. The open symbols (○, △) are for albino rats (two animals) and the closed symbols (●, ▲) are for a pigmented rat.

along a linear section obeying the Weber-Fechner law that $\Delta I/I$ is constant, and turned sharply upward. Because of its similarity to what Aguilar and Stiles (*6*) have termed rod saturation, I have used "saturation" when referring to this rapid decrease in sensitivity. Usually the intrusion of the second mechanism prevented the saturation process from being followed further than a 0.8 log deviation from the Weber-Fechner line. Saturation was measured on 12 rats and was found to start at -4.05 ± 0.5 S.D. log background. By using the calibrations of retinal illumination of Cone (*11*) it is possible to estimate the number of quanta per second incident on the retina at saturation. By direct photometry, if no filters were inserted in the beam the background luminance was about 3.4 log cd/m^2 on the inside of the Ping Pong ball. For a light of 500-nm wavelength this corresponds to 9 ± 0.5 log quanta mm^{-2} sec^{-1} incident on the retina at the start of saturation. This compares with 9.2 log quanta mm^{-2} sec^{-1} of 500-nm required to saturate the rods in man (*12*).

To observe rod saturation psychophysically Aguilar and Stiles (*6*) utilized the two-color threshold method. Likewise, when the ERG is used to monitor sensitivity in the rat, a short wavelength test stimulus superimposed on a long wavelength adapting field reveals saturation. The experiments reported here differ from the Aguilar and Stiles design only in using a

flashed adapting background. Substitution of a steady long wavelength adapting field did not appear to significantly alter the results. The rat increment threshold curves still showed rod saturation. The flashed background, however, simplifies the interpretation by excluding the possibility of attributing the effect to the exhaustion of rhodopsin in the rods.

The next experiment, aimed at identifying the pigments responsible for dark- and light-adapted rat ERG's, involved determining the flash energies needed to produce criterion responses at several different wavelengths. Spectral sensitivity measurements were made on the dark-adapted rat retina. These measurements are plotted in Fig. 3 where the solid curve is for a Dartnall nomogram pigment peaking at 500 nm. These determinations show that the dark-adapted retina has the sensitivity of ordinary rods. Next, the retina was light adapted with a flashed background adjusted to be 1 log unit above the lowest level of background which produces saturation. Light adaptation produces a second spectral sensitivity curve showing increased long wavelength sensitivity without a significant shift in the peak.

Many years ago Granit (*13*) measured the spectral sensitivity of single units in the light-adapted rat retina. His photopic spectral sensitivity curves had two maximums around 500 nm and 600 nm. This led to the hypothesis that cones filled with visual purple and cones with a long wavelength sensitive pigment were connected to a common channel which mediated the light-adapted responses. These notions are consistent with the findings reported here.

There is suggestive physiological evidence in the literature for two mechanisms contributing to flicker fusion of the rat ERG (*2, 3*). Previous experiments have shown a clear break in the curve relating flicker fusion frequency and light intensity. To examine the relationship between the findings reported here and the flicker fusion studies, a sectored disk driven by a variable-speed motor interrupted the light from the flashed background. The speed of disk rotation was varied to produce a 20-μv flicker response. The flicker fusion frequency increased only slightly (Fig. 2) over the first 3-log increase in stimulus intensity. At about −4 log mean intensity the flicker fusion frequency rapidly increased. The break in the flicker fusion frequency curve occurred at about the same intensity as the shift in spectral sensitivity.

In summary these findings suggest that there are at least two receptor mechanisms in the rat retina: a classical rod, and another mechanism differing from a rod in absolute threshold, spectral sensitivity, and speed of response. The presence of more than one receptor mechanism explains the discrepancy between the limited response range found for the human rod system and the great range of light adaptation exhibited by the rat retina.

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Calcium Carbonate Concretions: Cyclic Occurrence in the Hamster Vagina

Abstract. *Three crystalline forms of calcium carbonate were identified in washings of the hamster vagina. Spherical concretions of vaterite and hexagonal concretions of calcite predominate on days 3 and 4 of the 4-day estrous cycle. Dumbbell-like concretions of aragonite predominate during pregnancy and pseudopregnancy. Each polymorph is associated with an acid-insoluble matrix. Concretions disappear after ovariectomy and reappear during daily injections of estrogen and progesterone.*

It is common practice to follow the 4-day estrous cycle of the golden hamster (*Mesocricetus auratus*) by examining vaginal washings or smears daily for their cellular content. A massive discharge of nucleated epithelial cells suspended in mucin occurs every fourth day (*1*). We designate this as day 1 of the cycle. Under our laboratory conditions, ovulation occurs about 2:00 a.m. of day 1 and estrus begins about 9 hours earlier (*2*). Cellular changes characterizing each day of the hamster cycle have been described in detail (*3*), but the occurrence of noncellular calcareous concretions in the vagina has not been reported.

We observe these concretions in vaginal washings, obtained each morning with a dropper and tap water and examined while still wet under a microscope (× 100), on days 3 and 4 of the estrous cycle and during pregnancy and pseudopregnancy. They are absent on days 1 and 2 of the estrous cycle, during lactation, during acyclic periods due to

Table 1. Analysis by atomic absorption spectrophotometry of vaginal concretions collected on days 3 and 4 of the 4-day estrous cycle (E) and on days 5 to 15 of pregnancy and pseudopregnancy (P).

Sample source	Hamsters (No.)	Collections (No.)	Concretions					
			Micrograms	Micrograms per day*	Ca (%)	Mg (%)	Na (%)	K (%)
E	68	558	31.5	56	33.7	1.18	0.34	
E	34	218	11.8	54	33.9	1.24	.37	0.036
P	36	351	28.1	80	34.6	0.54	.27	
P	22	196	14.2	72	34.4	.53	.31	.028

* Micrograms of concretions produced per hamster per day.