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BLEACHED RHODOPSIN AND VISUAL ADAPTATION

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For many years all visual adaptation was supposed to depend essentially upon the state of bleaching and regeneration of rhodopsin—a doctrine called 'The Photochemical Theory of Vision'.

The visual adaptation was studied in man and naturally its relation to the bleaching and regeneration of rhodopsin remained unsatisfactory until this could be measured in man. Ten years ago Campbell & Rushton (1955) first studied this by retinal densitometry and showed that the rate of regeneration was of the right order to fit the dark adaptation curve, but could not possibly account for the rapid dependence of increment threshold upon background fields. Better work followed. Dowling (1960) obtained a good linear relation between the amount of rhodopsin bleached in the rat and the log threshold for its e.r.g., and Rushton (1961) showed the same relation for log visual threshold in man using a subject with a rare but simplifying condition in which rods are normal but cones nearly absent.

But the relation clearly established between bleaching and log threshold cannot be fundamental since it depends upon the kind of stimulus used for the threshold test. Many, following Craik & Vernon (1941), had found that the shape of the dark adaptation curve depended upon the area or other parameters of the test flash used, and Crawford (1947) had shown how to get a more unified conclusion by applying the principle of 'equivalent background' that had earlier been found valuable in the analysis of glare. In two of the preceding papers (Blakemore & Rushton, 1965*a*, *b*) we have confirmed and extended Crawford's results and concluded that when any region of retina is exposed to strong uniform light so that a fraction B of rhodopsin is in the bleached state, the adaptation produced is the same as that resulting from a luminous background I_B applied to the same retinal region.

If I_D is the receptor 'dark current' or '*Eigengrau*'

$$\log_{10}(I_B/I_D) = \alpha_B \qquad (1)$$

where $\alpha = 20$ in man. It was also shown that when an external background I is projected on the bleached region the real and equivalent

backgrounds add so the adaptation is the same as to a simple background of strength $(I+I_B)$.

The implication of 'equivalent background', however, is rather far-reaching. A recent paper (Rushton, 1965*a*) asked the question 'when the threshold is raised by a background, what is it that become less sensitive? Is it the rods that need to catch more quanta to be excited, or is it their summation pool that needs to receive more rod signals to detect the increment flash?' Two kinds of experiment strongly favoured the second alternative. A background exposed for 2 sec was so weak that only 1% of the rods on which it fell could absorb a single quantum. Yet its presence raised the threshold of a superimposed flash to three times the absolute threshold. Clearly the threshold of the 99 that did not 'see' the background was raised by the one neighbour that did. Again at higher levels when the background was striped by a grating (stabilized on the retina) and the flash was similarly striped, it was found that it made no difference to the increment threshold whether the two gratings were in-phase (so that bright bars fell on bright bars) or out-or-phase (bright on dark). The period of the grating was $\frac{1}{2}$ degree and thus fell within the receptive field of the summation pool.

It seems reasonable to conclude that rods themselves do not change in sensitivity but always send a message to the pool for every quantum caught. The total flux of these messages modifies the sensitivity of the pool and determines the sizes of flux-increment needed for detection. Thus the pool's sensitivity depends only upon the total flux received and not upon the region of receptive field from which it comes. So when we say that the bleaching of rhodopsin changes the retinal organization as does a luminous background we must imply that the threshold is raised not in the rods but in the summation pool. If this were true it would mean that bleaching one point in the pool's receptive field would raise about equally the threshold of all points in the field, bleached and unbleached alike. This suggestion was kindly examined and confirmed by Lipetz (1961) who showed, by records from a ganglion in an excised frog's retina, that the log threshold for a less excitable point A in the receptive field was raised more by bleaching an excitable point B than by the same bleaching of A itself.

A year later Rushton & Westheimer (1962) were able to reach the same conclusion in experiments on man. We bleached with an electronic flash in the path of which was either a grating (of period 1° sharply in focus on the retina) or a neutral density of 0·4 that was found to transmit the same total light. Following these bleachings the dark adaptation curve was measured with the grating in the test upon the uniformly bleached retina and with the 0·4 in the test upon the striped retina. The two dark adaptation curves were practically identical. Thus with bleachings as with back-

grounds, rods in the shade that have been spared exposure become as insensitive as their illuminated neighbours. It is not in the rods but in their summation pool that the change is situated, and that seems to depend upon the total bleaching of the receptive field and not upon the spatial distribution of light there.

We concluded above that a test flash falling on a region of bleached retina finds the threshold raised just as though it were falling upon a luminous background. But it actually *does* fall on what looks like a luminous background, for substantial bleaches leave a more or less bright positive after-image in the bleached region. Could that be the very background we postulate? This attractive idea seems hardly plausible. The after-image is so weak that many people are not even aware of its existence, but no one could overlook an external patch as bright as the 'equivalent background'—obviously the two are different orders of magnitude. Yet we must bear in mind that the after-image, being stabilized on the retina, will quickly fade and be judged an order dimmer than its external equivalent. Before dismissing the question out of hand, the after-image should be compared with a *stabilized* equivalent background.

Barlow & Sparrock (1964) have devised an elegant way of doing this and found the 'brightness' of the after-image in terms of the luminance of a stabilized external field that matched it. Not only was the brightness so measured found to fade throughout 30 min in a way that followed closely the rod dark adaptation curve, but the matching luminance had the actual value required of the 'equivalent background' as determined from increment thresholds.

From these striking results we are tempted to say 'Thresholds during dark adaptation are raised simply *because* the test flash falls upon the bright after-image as background'. One of the objects of the present paper is to help readers to resist this temptation.

METHOD

Increment thresholds and dark adaptation thresholds were made with the equipment described in Blakemore & Rushton (1965*a*) and shown there in Fig. 1. The subject was clamped in position by dental impression and brow rest and usually the pupil was dilated with some mydriatic. Test flash and background fields were presented in Maxwellian view. The flash was green, the field red (Ilford filters 624 and 205), and the intensities were adjusted by neutral filters and wedges. The flash was presented as a circular patch of diameter 5°, the field subtended 10° and the threshold was reached by the subject adjusting the wedge, or waiting in dark adaptation until a pre-set test flash became first visible. A red fixation point lay 5° temporal to the centre of the flash and background.

THE PRINCIPLE OF THE EXPERIMENTS AND THEIR RESULTS

The object is to show that the spatial integration of a luminous background of real light is very different from that when the luminous background is an after-image.

Increment threshold. In Fig. 1, curve *A* (open squares) shows part of an increment threshold curve obtained with a uniform green circle presented flashing upon a large uniform red circular background. The log thresholds (ordinates) are plotted against log backgrounds in scotopic trolands and the results are similar to those of Fig. 1 of the preceding paper (Rushton, 1965*b*) which was from the same series of experiments.

In the measurements of curves *B* and *C* the background field was not uniform but consisted of an array of luminous points. A metal plate was perforated by holes drilled upon a lattice of equilateral triangles, and this plate was placed in the background beam where it was uniformly illuminated and where the holes were in sharp focus on the retina, subtending an angle of about $\frac{1}{2}$ a degree from hole to hole. Consequently (as seen by cones) the background presented the appearance of an array of luminous points on a dark ground. There were two such plates, similar except for the size of the holes; in one about 0·3 of the metal was removed, in the other about 0·1. Consequently the two plates would cut down the total background light about as much as would a 0·5 or 1·0 filter, respectively. The actual equivalent densities were measured with a photocell and wedge in a uniform beam. Each plate was interposed in turn and the wedge shifted to restore the photocell output. The equivalent densities so found were 0·44 and 1·04.

Now, the increment threshold (curve *A*, Fig. 1) was repeated but with the 0·44 or the 1·04 perforated plate interposed in the background. Obviously if a neutral density filter of 0·44 or 1·04 had been interposed instead, the results would be identically curve *A* shifted 0·44 or 1·04 to the right—where they have been drawn as curves *B*, *C*. Curve *B* fits the experimental circles but the triangles run up less steeply than curve *C*. However, there is no suggestion that the extended uniform test flash, falling upon the dark spaces between the stars, finds the retina there near 'absolute threshold'; the results rather support the general rule (Rushton, 1965*a*) that thresholds are raised simply by the average light flux of the background independent of its distribution. Yet the substantial divergence of the triangles shows that this rule is not exact with small points of light—perhaps because points do not fall upon every summation pool, and those spared are more numerous or more effective at high intensities.

Dark adaptation. The retina was bleached by a 5 msec electronic flash seen in Maxwellian view with (or without) one of the perforated plates

interposed sharply in focus. This left a bright after-image that resembled precisely the backgrounds in conditions *A*, *B* or *C*, Fig. 1, as regards size distribution and position of luminous points on the retina. All rods that *were* bleached were bleached equally in the three conditions, and all presumably regenerated at the same rate. Only the spatial distribution was different, and different precisely as in curves *A*, *B* and *C*. Thus from the ordinary dark adaptation curve *D* together with curves *A*, *B* and *C*, we can predict the dark adaptation with an after-image of stars by application of the principle of 'equivalent backgrounds', if the after-image is in fact equivalent.

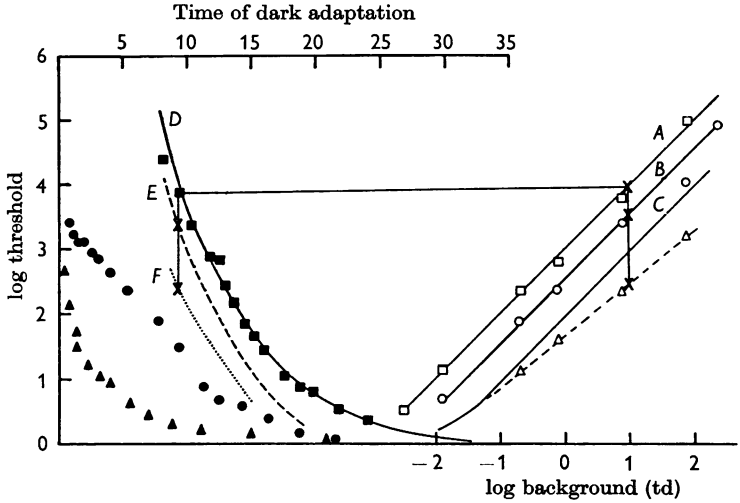

Fig. 1. Open symbols, increment threshold curves for rods, green flash on red background, abscissae (scale below) in log scotopic trolands. Filled symbols rod dark adaptation curves (scale above). Squares are when background and bleaching were uniform fields; circles when they were presented as an array of luminous points that covered $\frac{1}{3}$ area; triangles when the points covered $\frac{1}{10}$ the area. *E* should coincide with circles, *F* with triangles if after-images act like external background lights.

The dark adaptation curve after bleaching with a uniform flash is plotted by filled squares, curve *D*, Fig. 1 (time scale above). It has a range of 4·5 log units, determined by the technique described in the preceding paper (Rushton, 1965*b*) and the curve drawn through it is the exponential that falls to half value in 4·5 min (as in Fig. 1 of that paper).

Now take any points on curve *D*, say at 9·5 min; the threshold is the same as at 1 log td of background in the increment threshold curve *A*. Thus from Barlow & Sparrock (1964) we know that the after-image was 1 log td bright after 9·5 min of dark adaptation. When the same bleaching was seen through the perforated plate, then at 9·5 min the brightness would also be

1 log td, but distributed in a pattern of points as it was in conditions B or C. Thus the points on the dark adaptation curves E and F at 9·5 min should have the same log threshold as B and C at 1 log td. The method that has defined E and F at 9·5 min is equally applicable to all other times and so curves E and F are constructed. E lies nearly 0·44 below D at every ordinate since B lies 0·44 below A. F starts about 1·5 log units below D and ends about 1 log unit below in accord with the more gentle (dotted) slope of the actual triangles.

Now the experimental determination of dark adaptation following bleaching with the 0·44 plate is shown by the black circles of Fig. 1. They do not fit curve E at all. The points after bleaching with the 1·04 plate are the black triangles. They do not fit curve F at all. The misfit is so considerable as to demolish the idea that the luminous after-image always acts in the same way as a real luminous background. It clearly does not act the same with regard to spatial integration.

Indeed it would not be expected to do so if Rushton & Westheimer (1962) were correct in their conclusion that dark adaptation depends upon the average bleach but not upon its spatial distribution. Denote by y the ordinate of curve D, Fig. 1; it is the rise in log threshold above the fully dark adapted value A. Since the test flash was large in area we know (Rushton, 1961) that the relation of log threshold to the fraction B of rhodopsin bleached is

$$y = 20B. \tag{2}$$

Suppose now that the bleaching flash is exposed through a well-focused perforated plate where $\frac{1}{3}$ of the metal was drilled away in holes. Then only $\frac{1}{3}$ of the rods will be bleached but *they* will all be bleached to the extent B. Consequently the average bleach will be $\frac{1}{3}B$ and from eqn.(2) we should expect y in this case to be $\frac{1}{3}$ of what it was without the plate interposed. Turning to Fig. 1, the black circles are when this plate was interposed, curve D when it was not, thus the circles should lie on curve D scaled down to $\frac{1}{3}$. This is certainly better than curve E.

Figure 2 reproduces the black points of Fig. 1 and also curve D, which has the equation (in min):

$$y = y_0 10^{-t/15}$$

(since y falls to half value in 4·5 min).

The curve through circles and through triangles is curve D scaled down in proportion to the fraction of light transmitted through each perforated plate. The geometry of the exponential makes this scaling easy. The curve D has simply to be slid to the left $0{\cdot}44 \times 15$ and $1{\cdot}04 \times 15$ min where 0·44, 1·04 are log transmission through each plate.

Before 8 min of dark adaptation there is no curve D to be scaled. After

this time the scaling fits the points reasonably well, for the operation admits of no degree of freedom—the scaling of curve D is entirely determined by measuring the holes in the perforated plate.

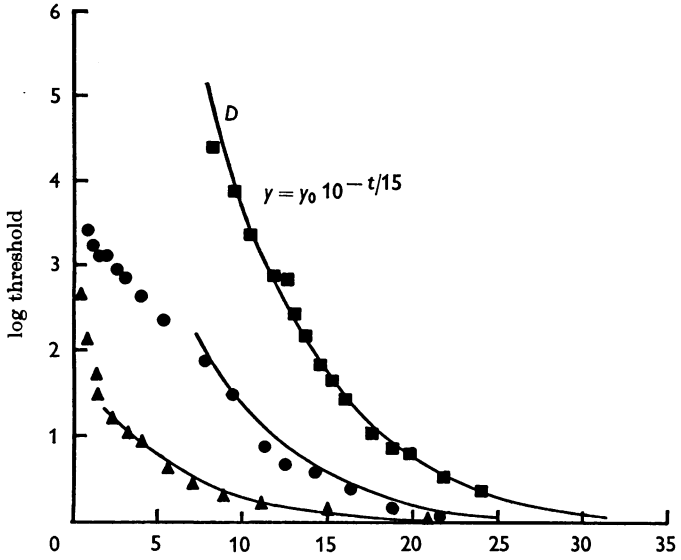

Fig. 2. Dark adaptation curves following a flash bleach with uniform field (squares) or an array of luminous points that covered $\frac{1}{3}$ area (circles) or $\frac{1}{10}$ area (triangles). Curve D is exponential of equation shown. Other curves are rescaling of D to $\frac{1}{3}$ and $\frac{1}{10}$ of the ordinate respectively.

DISCUSSION

The equivalent background of bleaching. This is a principle that has been considered twice in this paper. It was found valid in Crawford's (1947) experiments with test flashes of various kinds and in their extension by Blakemore & Rushton (1965*a*, *b*). But in the experiments of Fig. 1, where a real background was compared with a similar after-image, the equivalence was found false. It is important to be clear about the proper application of the principle, in which we may be helped by the very crude idea of adaptation indicated in Fig. 3.

Somewhere between the rods and the place where a threshold flash is detected is a rather complex mechanism of Gain control represented by the box G, Fig. 3*a*. A test flash ΔI will be detected if the output ΔV of the G-box reaches a critical level ΔV_0. We may write

$$\Delta V = G.\Delta I \tag{3}$$

thus, as G varies, so must ΔI vary inversely if ΔV is to remain at the threshold value ΔV_0.

Now the flash ΔI may be a test flash of any size and enter the G-box through all or only a few of the possible paths. We know from Crawford that the smaller the patch the less the threshold will be raised by a given increase in background. Consequently our model must change not only the gain but also the spatial integration. It might therefore be supposed that G is controlled by two kinds of 'knob', one to regulate the intensity factor and one to regulate the spatial factor. This is precisely what Crawford's equivalence shows is not the case.

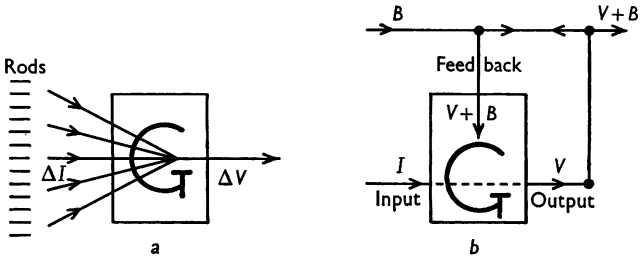

Fig. 3. Schema of retinal adaptation.

In terms of our model what Crawford did was to change G by bleaching and by backgrounds and to compare the organization of the G-box in the two cases using different types of test ΔI. He found that when the G settings matched by one test they matched by all. Intensity and spatial integration were not independent variables that needed separate matching adjustments. All the factors that G controls are the function of a single variable (one knob).

But Crawford's principle of equivalence that says 'bleachings and backgrounds act by turning the same knob' says nothing about their relative turning powers. The equivalence that generated the inappropriate curves E, F, Fig. 1, asserted something quite different, namely that bleachings and backgrounds are equivalent in their grips on the knob. This is entirely wrong; they both turn the same knob, but they do so in a very different way.

Connexions to the G-box. The principle of Rushton & Westheimer (1962) confirmed by Fig. 2 of this paper states that the effect of bleaching upon the summation pool depends upon the average amount of bleached rhodopsin in the rods connected to the pool independent of its distribution in the receptive field. For this to be true, the pool must somehow be able to receive continually a statement of the average residual bleach in its field. Thus in principle each rod must emit a signal proportional to its free opsin at the moment, these signals must be collected and something proportional to their sum transmitted to the pool.

The effect of the total bleaching signal B upon the gain G is found from eqns. (3) and (2) rewritten as

$$\log(\Delta I/A) = 20B,$$

$$\therefore \Delta I = A.10^{20B} = \Delta V_0/G,$$

or

$$\log \frac{1}{G} = 20B + \log(A/\Delta V_0). \tag{4}$$

The background I behaves quite differently. It probably enters the G-box by the same path as ΔI and its output V leaves by the path ΔV—for after all ΔI and ΔV are the increments in I and V. From Fechner's well-known relationship we should expect V to be more or less proportional to $\log I$, and ΔI at threshold to be kI where k is a constant. Combining this threshold condition with eqn. (3) we obtain

$$\Delta V_0 = GkI,$$

or

$$\log \frac{1}{G} = \log I + \log \frac{k}{\Delta V_0}. \tag{5}$$

Comparing eqns. (4) and (5) we see that B is related to $\log(1/G)$ not like I but like $\log I$. Thus, if I is to grip the G-knob as B does, it must first be transformed from I to $\log I$. But this is precisely the transformation we have just postulated between I and V—the change in fact that I undergoes in passing through the G-box. Thus V is the signal that can meet B on equal terms in sharing the control of the G-knob. If we suppose that the feedback from the output V adds to the bleaching signal B and that $(V+B)$ operates on the gain, not only will this explain our results but it presents us with an automatic gain control very similar in outline to the detailed model by which Fuortes & Hodgkin (1964) analysed so exactly the intracellular potentials from the eye of *Limulus*.

The connexions of our G-box are represented schematically in Fig. 3*b*. The flux I of rod signals, each due to the catch of one quantum, enters the box at input, suffers a quasi-logarithmic transformation due to the gain mechanism and emerges as V. This output is fed back towards G. The bleaching signal B monitors the average state of bleaching in the receptive field (i.e. the total quantity of free opsin). It adds to V and the combined signal $(V+B)$ has two destinations. It enters the feedback and controls the gain; it is transmitted to the brain and engenders the sensation of brightness whether of lights (from V) or of after-images (from B).

Without further assumption, this model allows us to derive quantitatively all the relations we have been considering in adaptation to bleachings and backgrounds, except for the effects of G on spatial integration. If anyone

is interested in this analysis he may find it in my Ferrier Lecture to the Royal Society (Rushton, 1965*c*).

SUMMARY

1. The relation between the bleaching of rhodopsin and the rise in rod threshold is reviewed. Bleaching an area affects adaptation as though a luminous background covered the area.

2. Bleaching an area leaves an after-image that Barlow & Sparrock (1964) have shown has actually the brightness of the 'equivalent background'. Are bleached rods then sending signals similar to those in response to a luminous background?

3. This attractive idea is shown to be quite wrong by using a background consisting of an array of luminous points, and comparing the spatial interaction when this background is an after-image or external light. With real backgrounds the rise in log threshold is the *log of the average* background; with after-images it is *average of the logs*—a very different thing.

4. The Discussion leads to the Schema of Fig. 3. A signal B, proportional to the average bleaching, regulates the Gain mechanism that controls the size of the output signal V for a given light signal I. The output also feeds back and adds to B, so that the gain is controlled by $(V+B)$. This signal, travelling also to the brain, gives rise to the sensation of brightness from light (V) and after-images (B).

I have gained immensely from discussions with Professor A. L. Hodgkin, in particular from his important concept that the bleaching signal acts directly upon the feed-back. My thanks are due, as usual, to Mr Clive Hood for all practical aspects of the work, and I gratefully acknowledge a grant from the U.S. National Institute of Neurological Diseases and Blindness (N.B. 03014–04).

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Note added in proof. Since submitting these four papers for publication I have become aware of a remarkable analysis by Stiles & Crawford (1932) that anticipates by thirty years my main conclusions. They point out that the concept of 'equivalent background' unifies the problem of visual sensitivity in two ways. First, it expresses by one variable the state to which the retina may be brought by backgrounds, by surround illumination, by the presence of a glare source, or by a past history of bleaching. Secondly it unifies the widely different results of tests with different parameters. For, the same equivalent background, however engendered, will give the same numerical result with a given test whatever its nature. These general concepts very carefully and exactly formulated are tested (and in the main supported) by a range of accurate experiments.