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THE DARK ADAPTATION CURVE OF RODS MEASURED BY THEIR AFTER-IMAGE

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SUMMARY

1. The common dark adaptation curve exhibits two branches; the course of the rod branch cannot normally be measured at early times since it lies above the observed cone thresholds. In this paper we measure it.

2. This is done by observing the negative after-image against a uniform background critically adjusted in luminance.

3. Adjacent to the bleached area to be studied is a second area more strongly bleached. If the background intensity is below threshold for the less bleached area it will not be seen there; but if the background is above that threshold, this area will be seen brighter than the other.

4. The dark adapted threshold on the less bleached area is therefore the background luminance which just permits the two areas to be distinguished in the after-image.

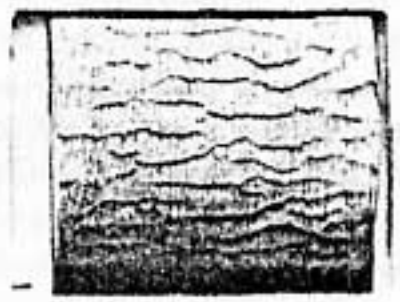
5. After 5 min cones have quite recovered, and thus have no after-image to contaminate the rod image.

6. The rod curve measured by after-image is traced over 5 units of log threshold: it is an exponential with half life of 4·5 min, and coincides with the time course of regeneration of rhodopsin in man.

INTRODUCTION

This paper concerns the measurement of the familiar dark adaptation curve by an unfamiliar technique.

The black circles of Fig. 1 show the familiar recovery of the log threshold following a light exposure that bleached away 60% of the rhodopsin in the annular region of the retina shown in the inset. After the light exposure, the eye remained in the dark and a 2° test light was flashed on to the annulus. The test flash was adjusted in intensity so that it could only



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just be detected (threshold), and the black circles plot log threshold against time in the dark.

The black circles lie on a two-branched curve, the early branch recording cone excitability that recovers fast and corresponds exactly to the regeneration of cone pigment (Baker & Rushton, 1965, Fig. 4). The later branch records rod excitability which corresponds to the regeneration of rhodopsin (Rushton, 1961, Fig. 1).

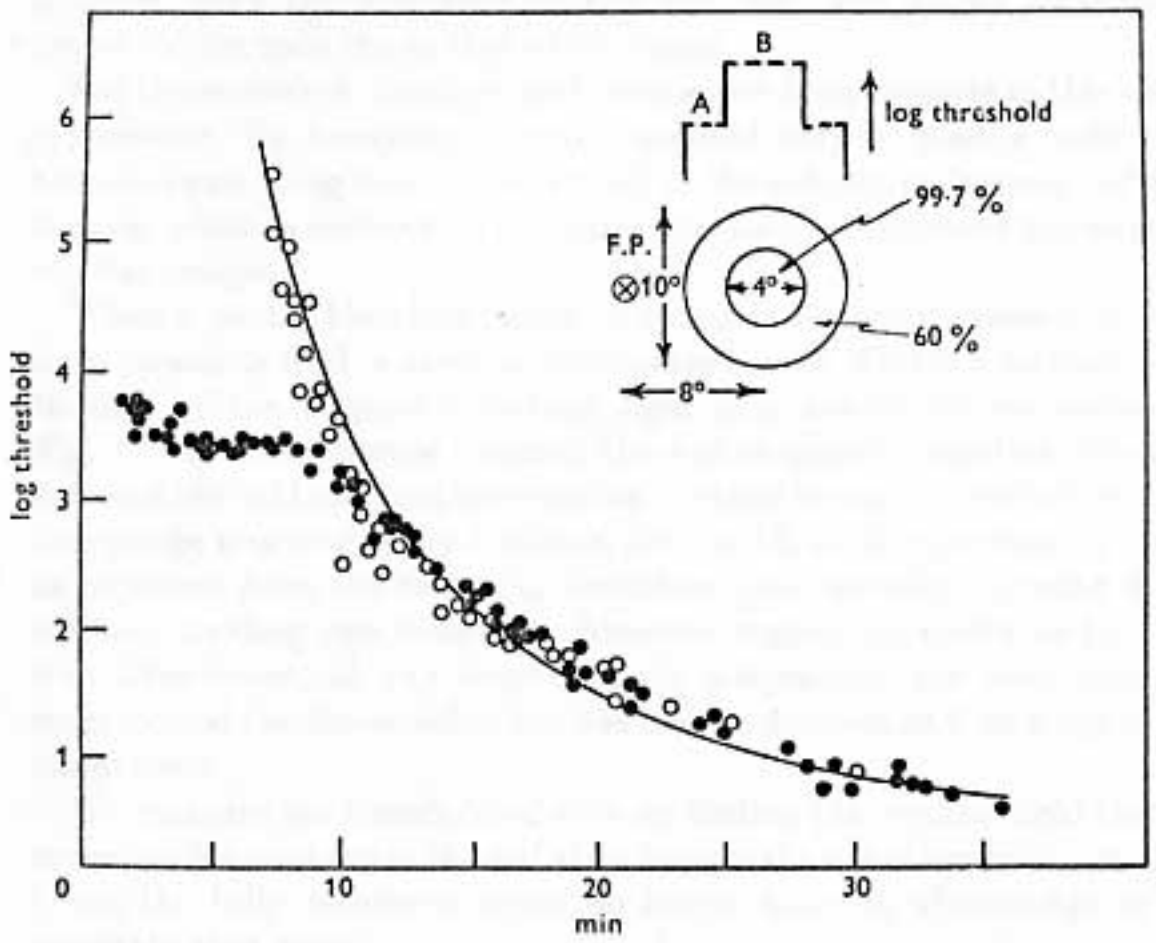

Fig. 1. *Inset.* The observer's view of the bleaching stimulus (= after-image). Amounts of rhodopsin bleached are indicated. Foveal centre shown by ×. Interrupted lines above indicate the level of log threshold on annulus (*A*) and centre (*B*) at some stage of dark adaptation. *Curves.* Black circles plot the usual dark adaptation curve, obtained by flashing a 2° test on to the annulus. White circles plot the log *background* flash just sufficient to reveal the halo around the black centre in the resulting negative after-image.

Rods and cones respond to the test flash independently, so the threshold observed is that which is lowest at the moment – that of cones at first because they are less incapacitated by bleaching and recover faster, that of rods later because their dark adapted threshold is much the lower.

Since this familiar threshold technique measures only the lower member of the two curves, it cannot define the course of either curve when it crosses the other and becomes the upper member. There seems no doubt

that the cone curve, which has already fully recovered in Fig. 1 before the crossing point, will continue to run horizontally to the right indefinitely, and on the fovea (in the absence of rods) this is seen to be the case.

With rods, on the other hand, two views have been entertained as to what happens above the cone threshold. Some have held that the rod curve continues to rise ever more steeply at shorter times like the white circles of Fig. 1, maintaining throughout the coincidence with the rhodopsin regeneration curve, as may be seen in the 'rod monochromat' with no cones to obscure the picture (Rushton, 1961, Fig. 1). But it is known that these subjects are 'dazzled' by daylight at about 1000 td, and some hold that lack of dazzlement in normal eyes is due to excessive rod signals being cut off by active cones. If this were true we might expect the rising branch of the rod curve to be cut off a little way above cone threshold. But we find (white circles of Fig. 1) that at short times the threshold continues to rise smoothly along the exponential curve, just as it does in the rod monochromat.

The new technique

This is a rather small modification of the old technique. Instead of the test flash being a small luminous patch presented upon some point in the bleached area to be studied (e.g. on the annulus in Fig. 1), it is a large uniform luminous patch covering the whole of the bleached area.

A second essential feature is that in addition to the bleached region studied (which in our experiment was the annulus with rhodopsin 60 % bleached) there must be a neighbouring region more strongly bleached (in our experiment the centre which was nearly fully bleached).

Consider the effect on rods of flashing upon the bleached area a uniform field of adjustable luminance. In our experiment this fell exactly upon the bleached area and did not overlap on to the unbleached area outside the annulus.

Above the inset of Fig. 1 the interrupted line indicates for some moment in dark adaptation, the log threshold for rods, high at centre (*B*), lower on the annulus (*A*).

Suppose the log intensity of the large test field were less than *A*, the rod threshold for the annulus; then none of the rods that had been bleached could 'see' the flash (and none except those received the flash). Hence that flash would go undetected by the rod system. If the log flash intensity lay between *A* and *B*, the rods in the annulus would be excited but the centre not. The flash would therefore exhibit a black centre surrounded by a luminous halo. It is plain that the threshold for rod-perception of the halo *is* the dark adaptation threshold for rods there.

We must also consider the contribution of cones to the visual effect, and

this is simplified by the fact that our measurements do not start until after some 5 min of dark recovery. At this time cones have completely recovered and the uniform test field falls on a uniform cone population.

Late in dark adaptation when the rod threshold lies below the cone threshold, naturally cones contribute nothing and rods only are involved, as usual. The advantage of the new technique appears when the test field lies above the cone threshold. Here the uniform cone field does not interfere with the detection of the rod halo, and so we measure the threshold for rods above that of the cones.

For those readers who have not themselves been subjects in this kind of experiment, the foregoing objective account may be readily understood, but the same thing can also be stated in the subjective language of after-images, which is more vivid for those who have experienced the sensation of after-images.

When a partly bleached region of retina is suddenly exposed to a uniform luminous field, a *negative after-image* is seen. This is a picture similar to that of the original bleaching light (e.g. the centre and annulus of Fig. 1) but with reversed contrast like a photographic negative. The luminance of the field required to reveal the contrast in various parts of the negative image was studied by Rushton (1971*a*, *b*), with experimental results as expected from the foregoing considerations, namely, in order that a contour dividing two differently bleached regions be visible in the negative after-image, at any stage of dark adaptation, the field luminance must exceed the threshold of the less bleached region at that stage of dark adaptation.

We measure the threshold of rods by finding the weakest field that will reveal such a contour in the rod after-image (the halo threshold), and after 5 min the fully recovered cones no longer have an after-image to contaminate that result.

METHODS

In fact, this experiment was part of a series to be described in a later paper and we used the rather complex arrangements of that work. Bleaching and viewing were with a stabilized image produced by a target borne on a contact shell. Since, however, most of the complication is irrelevant to the requirements of the present experiment, we shall here simplify the description.

Bleaching. The bleaching light was presented in Maxwellian view to the subject with head clamped by dental impression with target position adjusted so as to bring the bleached area to a retinal region 8° away from the fovea. The light passed through a gelatine sheet of neutral density 0·8 with a hole punched in it; the sheet was so placed that the hole was seen in sharp focus. The bleaching energy delivered through the hole was 7·7 log td sec, through the filter 6·9. The table give on p. 1078 of Rushton & Powell (1972) shows that these exposures bleached 99·7 and 60% of rhodopsin respectively. The resulting after-image had the form shown by the inset of Fig. 1.

Our electronic flash was not strong enough to deliver these energies. We used a steady light with 2 sec exposure, the stabilized target avoiding smudging by eye movement.

Dark adaptation curves were obtained (*a*) by the conventional method and (*b*) by after-image discrimination.

(*a*) A test flash of area 2° lasting 0·2 sec was projected on to the region bleached by the annulus, and was repeated at 1 sec intervals. The subject adjusted the light intensity throughout the course of dark adaptation so that it was just at threshold.

(*b*) In other runs this small flash was not used but instead a uniform background was repeatedly presented 1·5 sec on, 1·5 sec off and the subject gradually increased the intensity to the level I where the after-image first showed its *annular* structure. The background was blue-green in colour (Ilford 603 filter), and was a stabilized circle concentric with the annulus and extending nearly to its outer rim, so the unbleached area beyond was not illuminated. The background threshold, plotted as log I against time, gives the second kind of dark adaptation curves.

RESULTS

Fig. 1 shows the two dark adaptation curves (*a*) black circles by the conventional method, (*b*) white circles by after-image discrimination (halo detection).

At times greater than 10 min, white and black circles coincide. Consequently, when rods only are concerned, our conjecture is justified: the annulus can be seen distinct from the centre if and only if the threshold of the annulus is exceeded. The after-image measurements began after an interval of 5 min. As can be seen from the black circles, by 5 min cones had fully recovered, and attained a threshold well below that of the rods so that rod thresholds cannot now be measured by the conventional procedure (black circles) which only plots cones. But fully recovered cones present no after-image so they cannot at this time contaminate rod thresholds measured by the new procedure. The only after-image now to be seen is from rods.

To confirm that the late after-image originated from rods and not from cones, we bleached the fovea with a scaled-down version of the centre-annulus display, in which the centre subtended 1° 30′ and fell upon the fovea. In the resulting cone after-image, centre and annulus were at first distinguished only faintly, and were never distinguishable after 2 min. The indistinctness and short life-time of the cone after-image are partly due to the cones' greater photosensitivity, which allowed even the less intense annulus to bleach about 99% of the cone pigment; Brindley (1959) has previously observed that when two areas have had nearly all their pigment bleached, their after-images are indistinguishable. In parafoveal vision, the rod origin of the prolonged after-image with which we are here concerned has also been demonstrated by MacLeod & Hayhoe (1974) using a 'silent exchange' technique.

The white circles (Fig. 1), show the time course of log threshold, measured through a range of 5 log units from its starting point at more than a hundred times cone threshold, and lying throughout close to the exponential curve shown, of half-life 4·5 min. This is the normal recovery curve for human rods (Hecht, Haig & Chase, 1973, half-life 4·75 min, measured over 3 log units; Rushton, 1965, half-life 4·5 min, measured over 4·3 log units; Alpern, Rushton & Torii, 1970, half-life 4·5 min, measured over 6 log units, with rhodopsin regeneration coinciding).

We conclude that after-image discrimination is a valid method of measuring the dark adaptation curve.

DISCUSSION

The white circles of Fig. 1 plot the dark adaptation curve for rods using the 'halo' in the after-image. The results show that late in dark adaptation these thresholds coincide with the black circles, the log threshold obtained in the usual way. These lie on an exponential curve of half-decay time 4·5 min, which corresponds to the course of regeneration of rhodopsin, measured by retinal densitometry.

Towards early times in dark adaptation, this exponential rises rapidly and the rod thresholds (white circles) are seen to follow it, and hence to lie much above the log threshold for cones (black circles at 5 min).

Thus, though in this domain the rods have just the threshold expected from their rhodopsin content, it cannot be measured by the usual technique. For that notes the threshold for the most excitable mechanism which here is cones not rods.

However, the continuous regular rise in rod threshold above the cone level shown in Fig. 1 confirms results by three other methods.

(i) Rod-monochromats whose cones are few and rhodopsin-filled, may show the rod-branch only, in the dark adaptation curve, running as indicated by the white circles of Fig. 6 (Rushton, 1961).

(ii) If the rod threshold is measured by a green flash on a red background as Aguilar & Stiles have shown, the cone threshold is elevated, and rod thresholds may be measured above the level where cones normally intervene. Rushton (1965) applied this to the rod dark adaptation curve, which in this way was measured over 4·3 log units, and exhibited a curve like the white circles of Fig. 1.

(iii) Using Alpern's (1965) contrast-flash technique, Alpern *et al.* (1970) measured the dark adaptation in normal man over a range of 6 log units and showed that the log threshold-rise throughout was proportional to the fraction of rhodopsin bleached. The same exponential curve was again obtained.

Here are four methods of preventing the cones from 'underselling' the rod threshold. In (i) there *are* no low threshold cones; in (ii) the cone threshold is raised by a strong steady red background; in (iii) the cones are strongly excited by the contrast flash which is seen coloured, but on account of the high specificity of contrast-flash interaction, these cones do not raise the threshold for rods. Consequently, when a flash at centre that excites only rods has its threshold raised, it must be the result of *rod* excitation in the surround, independent of cone activity there.

(iv) The results of Fig. 1 are similar in principle to (iii). The background excites cones, but since that excitation can produce only changes in the *cone* after-image, and since that has completely faded after 5 min of recovery, the cones cannot affect the appearance of the rod image that is the basis of the measurement.

The curve of white circles runs up to 5 log units above rod absolute threshold. It stops here not because stronger flashes were unavailable, but because at earlier times no stronger flashes seemed effective. This confirms earlier measurements both on normal eyes (Alpern *et al.* 1970, Fig. 4) and on a rod monochromat (Rushton, 1961, Fig. 2). When bleaching has raised the threshold about 6 log units, which means about 50% rhodopsin bleached, the rod mechanism seems to have become totally unresponsive.

We have argued that since the recovered cones have no after-image, they cannot contaminate thresholds measured on the after-image of rods. It might be objected that the cones still 'see' the bright uniform field. Might not this perception raise the threshold for halo-detection by rods in the same way that a bright uniform background raises the rod threshold for a superimposed flash?

But, as Stiles (1939) has shown, the threshold for rods is raised by a background only to the extent that this excites rods; its excitation of cones is without effect upon the rod threshold. Alpern (1965) showed the same rod-cone independence in his after-flash interaction, and Hallett (1969) showed it using flashed backgrounds (though see Makous & Boothe, 1974). We thus should expect in our experiment that the threshold for halo-detection by rods should depend upon the stimulation of *rods* by the test background, and that the stimulation of cones should not affect it. That is what we have argued above.

Like the earlier measurements of rod dark adaptation in the normal eye, Fig. 1 shows recovery in the normal eye following exactly the same course as in the rod monochromat (Rushton, 1961), even though in the conditions of Fig. 1 cones were excited at up to 100 times threshold while rod threshold was measured. This does not support the conjecture that active cones 'turn off' rod signals.

Why, then, are rod monochromats dazzled in bright light, but normals

not? We know from Aguilar & Stiles (1954) that at a background luminance of about 1000 td rods saturate, i.e. even a very strong superimposed flash is not detected. Evidently there is an upper limit to the rod output, and if this is reached already by the background, the added flash can do no more. Consequently a daylight scene in which the less bright regions already exceed 1000 td must saturate rods everywhere, and all rod contrast will vanish. A subject whose retina contains only rods will experience something like entering a bright, homogeneous, thick, luminous mist through which no detail can be made out. He is sometimes described as 'photophobic' but he does not find light painful; he is just irritated not to be able to see things in the scene before him, and he 'screws up his eyes' (nearly closing the paplebral fissure) to reduce the retinal illumination. Better still, he puts on the dark glasses he generally carries, and by reducing the illumination to below 1000 td he restores contrast.

Doubtless the rods of normal eyes behave just like this in sunlight, but we can ignore their empty and uniform glare, for through it we can see what interests us – the contrasted and coloured picture from the cones.

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