J. Physiol. (1959) 149, 303–317

ROD–CONE INTERACTION IN THE FROG'S RETINA ANALYSED BY THE STILES–CRAWFORD EFFECT AND BY DARK ADAPTATION

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(*Received* 4 *May* 1959)

In the previous paper (Donner & Rushton, 1959) records were obtained from selected single ganglion cells, and it was found possible to substitute light of one colour for light of another without ganglion discharge if their relative light intensities were suitably adjusted. A somewhat unexpected situation arose in the mesopic range, where the spectral sensitivity did not correspond to rhodopsin alone, for there was a 'hump' of increased excitability in the green and often an additional 'hump' in the blue, but still the condition of silent substitution could be obtained. This state of affairs could be explained either by assuming a change in two screening pigments situated in front of the rhodopsin rods which alone were active, or by supposing that three different photoreceptors contributed to the visual response in this range. It is the chief object of the present paper to distinguish between these alternatives by showing, for instance, that cones are responsible for the green hump of the mesopic curve.

The usual way of distinguishing rods from cones is by noting the spectral sensitivity and the level of adaptation. Neither of these may be convincingly used in the present problem, but we have been able to employ a powerful tool forged by Stiles and his colleagues, the value of which has been strangely overlooked. They showed in man that light is much more effective when it falls upon the cones perpendicularly than obliquely, but that rods are nearly indifferent to the incident angle. We have been able to substantiate this relation for rods and cones in the frog, and to employ it in order to discover whether or not cones are involved in a given range of spectral sensitivity.

PART I

In 1933 Stiles and Crawford discovered the retinal direction effect which bears their names. A narrow pencil of light was sent into the human eye and brought to a focus upon the fovea. When the light passed through the periphery

of the dilated pupil it appeared much dimmer than when it passed through the centre, so that its energy had to be increased some three times to look equally bright. This was surprising, since the same amount of incident light was falling upon the same retinal region in the two cases. The suggestion that the peripheral rays suffer greater absorption has never been seriously entertained, for (as the authors worked out) the crystalline lens is the chief absorbing medium and peripheral rays have the shorter path through this structure. An even more convincing argument against differential absorption appeared when Stiles (1939) and later Flamant & Stiles (1948) showed that in a given small area of parafoveal retina the cones exhibited the Stiles–Crawford effect but the rods did not. This clearly points not to transmission losses but to a retinal direction effect to which the cones but not the rods are sensitive. That conclusion is confirmed in the present paper, where the effect is seen in the cones but not the rods of the opened, lens-free eye of the frog when the beam of light impinges directly upon the exposed retina from various angles.

Roughly speaking, two types of explanation have been advanced as to why oblique rays should be less effective upon the retina than normal rays. The least spectacular is the original tentative suggestion of Stiles & Crawford (1933) and Crawford (1937) that some screening material may lie between the receptors, as is certainly the case in the light-adapted frog's retina. As they pointed out, there is no histological support for this in the mammalian eye, nor would it be expected to discriminate so sharply between cones and rods. The alternative suggestion, which was first advanced by Wright & Nelson (1936), is that light must enter a cone axially to be effective for vision, possibly because these rays are kept in the cones by total reflexion. The actual mechanism of light funnelling has received considerable attention (O'Brien, 1946, 1947, 1951; Platt, 1947; Le Grand, 1948).

The most attractive treatment of this idea is that of Toraldo di Francia (1949), where each cone and rod is considered as a microwave antenna. It is well known that the most efficient type of receiver for detecting radiation incident along a fixed narrow beam is one constructed with directional sensitivity matched to the angular dimensions of this beam. High receptivity in the selected direction is associated with low reception for radiation at other angles, and the sensitivity falls rapidly outside the receptive angle. Now the observed Stiles–Crawford effect may be plotted upon a polar diagram, with the radius vector equal to the sensitivity for various angles of incidence upon the retina. When this is done, not only does this curve resemble the polar sensitivity diagram for a dipole radio antenna, but it looks as though the cones are directional receivers matched to fit the aperture of the daylight pupil and the rods are matched to fit the aperture of the twilight pupil. Thus the peripheral rays from a dilated pupil will fall outside the angle of reception for cones but not that for rods, thereby exhibiting the Stiles–Crawford effect with cone vision only.

Now, though the Stiles–Crawford effect has so far been investigated only in terms of human sensory judgements, electro-physiological techniques permit it to be studied also in animals, where important information can be obtained in relation to the size and shape of the pupil and the geometry of rods and cones, which differ very greatly among vertebrates (Walls, 1942). More important for our present purpose, the retinal direction effect might constitute a powerful tool in separating the contributions of rods from those of cones in a mixed situation, as has been already achieved in man by the elegant analysis of Flamant & Stiles (1948).

In the previous paper (Donner & Rushton, 1959) we recorded spectral sensitivity curves in mesopic conditions and found a hump of increased sensitivity in the green. This could be due to some contribution by the cones, or to some change in screening pigment in front of the rods which alone might be suppposed to be active at this level of adaptation. The Stiles–Crawford effect would distinguish between these two alternatives if in the frog, as in man, the retinal direction effect applies only to cones and not to rods. For we have only to see whether the green hump is direction-sensitive or not to know whether it is cone-dependent or not. The object of Part I of the present paper therefore is first to investigate whether the Stiles–Crawford effect does apply to the frog and, if so, whether to the cones only; and secondly to take up the question of the humps in mesopic sensitivity and to find to what extent these are cone-dependent. A brief note upon the Stiles–Crawford effect in the frog has already appeared (Donner & Rushton, 1956).

METHODS

Optical arrangement

The apparatus was a modification of that described in the previous paper (Donner & Rushton, 1959). Its modified form is shown in Fig. 1. Light from the 6 V bulb, A_1, was reflected by prisms and focused by lenses, L_1 and L_2, upon L_3. The two pathways were united at the thin glass plate M_1 by reflexion and transmission, respectively. The Maxwellian lens L_4 focused the filament image upon L_5, which replaced the crystalline lens of the frog and formed upon the retina, R, an image of the iris stop D. Thus upon the retina a circular, evenly illuminated field was obtained the size of which was determined by the iris stop, the diameter being 1/10 that of D. In the present experiments path 1 was usually cut off completely and only path 2 used. The intensity of light was controlled by the compensated wedge W and the range changed by neutral filters F_2 and F_3. Approximately monochromatic light was obtained from twelve interference filters mounted upon a wheel at C_2, as described in the previous paper. A photographic shutter, S, behind the filter-holder F_2, was used to give flashes of 1 sec duration. In order to be able to vary the angle of incidence of light upon the retina the lens L_4 was mounted upon a horizontally moving holder with micrometer adjustment and scale. A shift of L_4 to one side displaced the image of the filament on L_5 but did not affect the field upon the retina except that the direction of its incidence was altered. The dotted lines in Fig. 1 show the passage of light for a different position of L_4. In actual practice it was convenient to place the eye upon its back looking upwards and the rays leaving M_3 in Fig. 1 should be deflected by a mirror into the paper, thus causing them to fall approximately vertically downwards upon the retina.

The arrangement at the eye is shown in Fig. 2*a* where *M* is the micro-electrode with its tip upon the retina. By moving the lens, L_4 (Fig. 1), it was possible to vary the incidence of the light along *AD*, or *BD*, or any intermediate position. The dimensions were *CD* 25 mm, *AB* 10 mm. Thus the angle *ADB* was about 22°. Inside the retina this angle would be reduced to about 17° owing to refraction at the air-water surface. In order to get a wider range of variation of the angle of incidence from the perpendicular, the eye was often placed so that either *AD* or *BD* gave an approximhtely normal incidence on the retina.

The image of the filament which L_4 formed upon L_5 is shown in Fig. 2*b* where L_5 is seen from the top instead of from the side. The filament image was about 10 mm long and 1·5 mm wide and moved across L_5 in a direction perpendicular to its length. In order to admit only a constant narrow pencil of this light to fall upon the retina, L_5 was covered by a black card *Q*, perforated

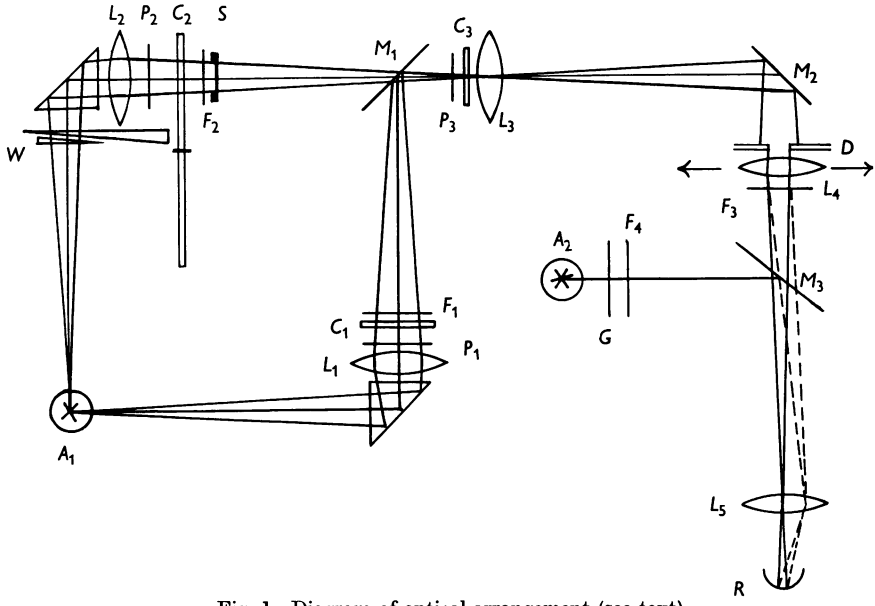

Fig. 1. Diagram of optical arrangement (see text).

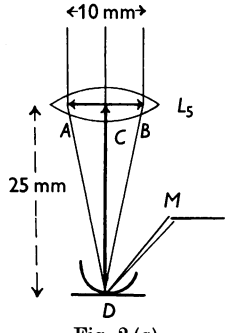

Fig. 2 (*a*)

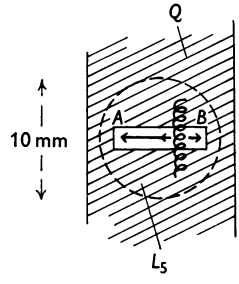

Fig. 2 (*b*)

Fig. 2. (*a*) Diagram of light falling upon a fixed point *D* of the retina from various directions *A*, *C*, *B*. *M*, micro-electrode. (*b*) *Q* is an opaque card placed over the lens L_5, with the slot *AB* cut to admit 2 mm length of filament image no matter where this lay between *A* and *B*.

by a slit 2 mm wide placed at right angles to the filament image. In this way whatever the position of the image upon L_5 only a fixed 2 mm length acted as light stimulus.

The calibration of the lights has been described in the foregoing paper (Donner & Rushton, 1959). In the present work the polaroids were removed. Their spectral density was found to be constant to within 0·05 throughout the visible spectrum, so their removal constituted a negligible disturbance to the former calibration.

To maintain a steady level of photopic adaptation, a constant, diffuse, white adapting field was superposed upon the retina. It was obtained by an ordinary 220 V, 40 W bulb, A_2 (Fig. 1) placed in a box behind a milk-glass screen G, and neutral filters F_4. The glass plate M_3 reflected the light into the eye through L_5, and thus the adapting field was independent of changes in angle of incidence of the stimulating beam.

It was convenient to know the position of the lens L_4 at which the beam fell perpendicularly upon the retina. This was found as follows. The lamp A_2 with its attachments G and F_4 was lifted aside, and the glass M_3 was used to observe reflexion from the retinal surface. It was easy to see both the image of the filament formed upon L_5 and the reflexion of light from the retina. L_4 was moved until these two coincided, in which condition the ray reflected from the retina retraced its path.

If the light falls upon the retina from various directions, in general the shadow of the micro-electrode will fall upon a different set of receptors. In order to avoid variation from this cause we usually arranged the light not to fall upon the electrode at all, stimulating only a part, but fixed part, of the receptive field of the ganglion. The same results were found, however, when the electrode was in the middle of the illuminated field, provided that it lay in the plane ABD (Fig. 2A), for in that case change of angle of the light did not produce any movement of the shadow of the electrode.

The frog's eye was dissected out and set up as described in the previous paper (Donner & Rushton, 1959) and the conditions for obtaining records from single ganglion cells were similar.

RESULTS

After dissection the eyes were set up and allowed to become dark-adapted for 1–2 hr. Then a ganglion cell was found which gave a good isolated discharge and threshold measurements were made with light at various angles of incidence upon the retina.

The results of one such experiment are given in Fig. 3 when the receptors from a single ganglion were excited by light of wave-length 516 mμ in various states of adaptation. Curve A shows the absolute threshold after 2 hr 15 min dark-adaptation. The log. sensitivity shows very little change with incidence. Curves B and C give the increment threshold superposed upon a white adapting field which in C was 10 times as strong as in B. The sensitivity is 2–3 log. units lower and there is a more pronounced dependence upon the angle of light incidence. Curve D is after 1 hr further dark-adaptation, and both sensitivity and dependence upon angle are intermediate between A and B.

The dependence of sensitivity upon angle was often greater than the results in Fig. 3. Under these experimental conditions the total threshold change for varying angles of incidence was between 0·4 and 0·8 log. units for different elements, the average of 13 being 0·49. Maximum sensitivity was not always at the point where rays fell perpendicular to the retinal surface as judged by

reflexion. The deviation was often 5° and never larger than 10°; but as it is very easy to deform the receptor layer by slight pressure of the electrode, such deviations are not surprising.

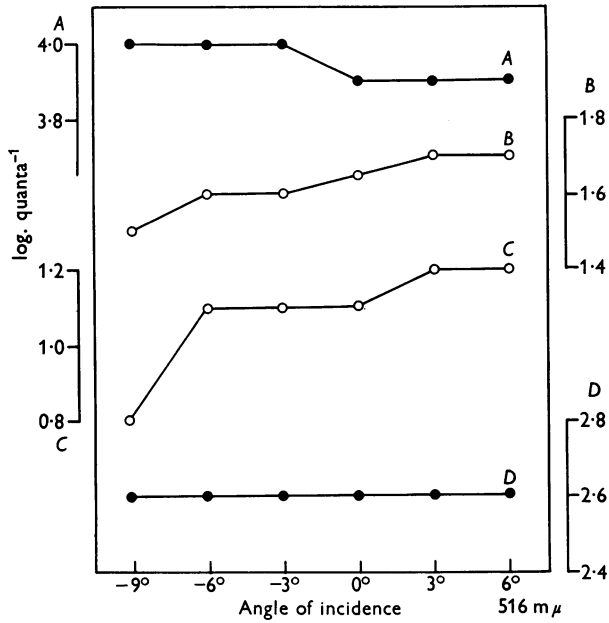

Fig. 3. Dependence of log. sensitivity upon angle of incidence upon retina. *A*, absolute sensitivity after 2 hr dark-adaptation. *B*, increment sensitivity against white background. *C*, as *B* but with background 10 times as bright. *D*, as *A* after 1 hr of dark-adaptation following *C*.

Spectral sensitivity

The results of Fig. 3 indicate that the retinal direction effect is well marked only in photopic conditions. More precise information as to the receptors responsible was obtained by plotting the spectral sensitivity to lights incident at the two different angles in scotopic and photopic levels of adaptation. Figures 4 and 5 are typical of a large number of experiments of this kind.

In Fig. 4 curve *A* shows the scotopic sensitivities measured by rays which fell more or less perpendicularly (circles) and at 22° from vertical (dots) measured in air, which is 17° from vertical in water. The curve is the absorption curve for frog's rhodopsin (Dartnall, private communication). It is seen that in this case the scotopic sensitivity is determined by the rhodopsin-containing rods, and that there is no retinal direction effect. This ganglion cell was then light-adapted by using the white adapting field from A_2 (Fig. 1) and the sensitivity was measured by superposing lights of different colours to obtain the increment threshold, which is plotted in *B* (Fig. 4). The spectral sensitivity had now shifted to the photopic dominator (lower curves *B*) and in contrast

to the scotopic state the direct ray (circles) is about 0·4 log. units more effective than the oblique ray at 17° (dots), this direction effect being nearly constant throughout the spectrum.

There are two possible interpretations. One is that the photopic dominator curve involves cones which (like human cones) exhibit a retinal direction effect. The other is that there is no retinal direction effect in the receptors themselves, either in curve *A* or curve *B*, but that in the photopic state black granules of the pigment epithelium wandered between the photoreceptors,

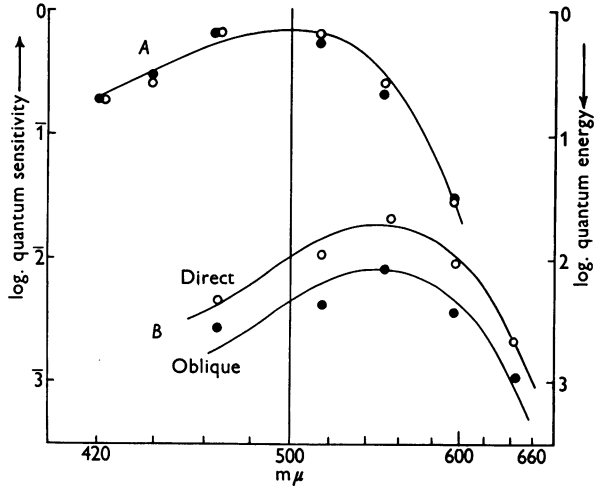

Fig. 4. Spectral sensitivity of Stiles–Crawford effect in opened frog's eye. Abscissae, wave-length of light on scale linear for frequency: ordinates, log. quantum sensitivity. ○, perpendicular incidence; ●, oblique incidence (17°). *A*, dark-adaptation, with rhodopsin curve and no directional effect; *B*, light-adaptation with photopic dominator curves and 0·4 log. units of directional effect.

and partly cut off the light which falls obliquely. It is unlikely that the difference between curves *A* and *B* of Fig. 4 is due to pigment migration, for according to Walls (1942, p. 152) no pigment change occurs when a light-adapted excised eye is left in the dark, as in our experiments. In confirmation of this, the frog's retina, dark-adapted in life, is pink and it is easily pulled out with the vitreous, whereas the light-adapted retina is black and well attached to the choroid. This latter condition was always found with our retinas, kept 2 hr in the dark after the eye had been excised and opened in bright daylight, though this was the condition which gave curve *A*, Fig. 4. A more convincing confirmation follows from the results of Fig. 5.

In that experiment white circles show the threshold sensitivity for perpendicular light, black circles for oblique light, and the continuous curve gives the absorption spectrum of rhodopsin. The adaptation level was intermediate between the full dark-adaptation of Fig. 4*A* and the full light-adaptation of

Fig. 4*B*. It is a mesopic level corresponding to Fig. 5 of the previous paper (Donner & Rushton, 1959), where the sensitivity curve was found to depart from that of rhodopsin by a hump in the green, and another in the blue. That these two elevations were due to separate *causes* was already clear from the fact that the green hump was present sometimes with, and sometimes without, the blue hump. That they are due to separate *receptors* is now clear from Fig. 5, since the green hump is direction-sensitive whereas the blue hump is not.

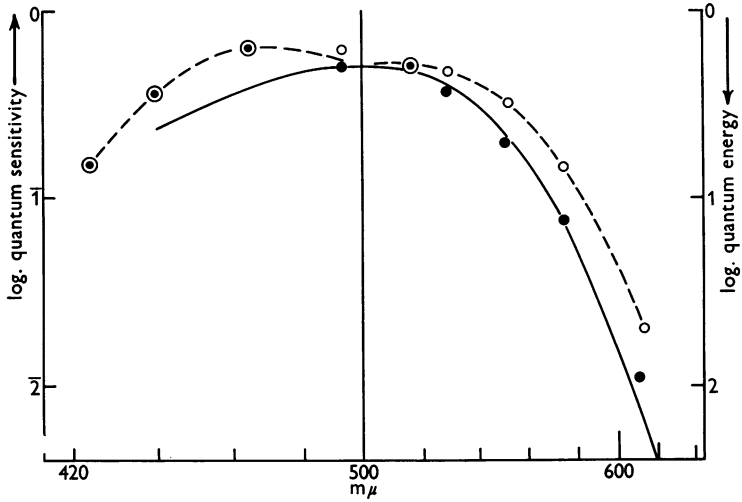

Fig. 5. Mesopic spectral sensitivity plotted as in Fig. 4. ○, perpendicular, ● oblique incidence of light on retina. The green-sensitivity hump is direction-sensitive and hence involves cones, the blue hump is not direction-sensitive and hence does not involve cones.

Since there is no question of pigment movements as between one part of the curve and the other, it is plain that the 'green receptors' are direction-sensitive and the 'blue receptors' are not, and we conclude that the hump in the green is due to the contribution by cones to the excitability curve. In Fig. 4 the change from direct to oblique light caused the recorded cone excitability to be depressed by about 0·4 log. units at all wave-lengths, so we might have expected in Fig. 5 to find a similar depression in the whole curve to the right of 500 mμ. Instead of this, the cone curve is replaced by an excitability corresponding closely to the rhodopsin absorption curve (full line), so the cones have evidently been depressed to a point where rods alone appear active.

The hump in the blue, on the other hand, does not involve cones, since it does not exhibit the Stiles–Crawford effect. It is therefore understandable that its presence is not tightly coupled with the presence of the cone hump in the green. The receptors responsible for the blue hump are not the rhodopsin rods, as may be seen from Fig. 6. The circles here give the spectral sensitivity

after 30 min adaptation to green light of 527 mμ (see upper arrow). The dots give the sensitivity after 10 min further adaptation to blue light of 438 mμ (lower arrow). In both cases the adaptation light was extinguished during the measurement of spectral sensitivity. It is clear that the blue hump is removed by adaptation to a powerful blue light. From these results one might infer that there was, in addition to the rhodopsin rods, a second kind of receptor which was not a cone and which was specifically sensitive to blue light.

Now exactly such a receptor has been known from the very outset of the studies on visual pigments (Boll, 1877; Kühne, 1878) and 'grass green' rods mixed with the purple population are shown in coloured figures by both these

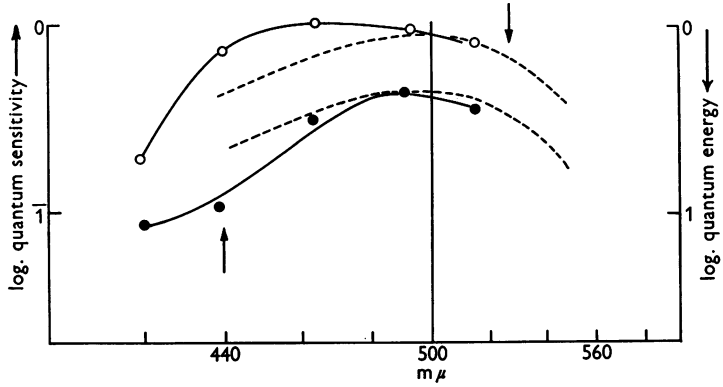

Fig. 6. Spectral sensitivity of the mesopic blue hump: ○, when strongly adapted to 527 mμ (upper arrow), ●, adapted to 438 mμ (lower arrow). Blue depresses but green does not, as would be expected if blue hump was due to the 'grass-green' rods.

authors. Recently Denton & Wyllie (1955) have published excellent photographs, not in colour but taken through suitable filters to bring out the relative densities of the two kinds of rod in green and blue light. According to the investigations of these authors the pigment of the green rods absorbs light of both red and blue wave-lengths, but only the blue rays are effective to produce bleaching. Green rays are not absorbed.

Physiologically, then, we might expect the grass-green rods to become 'dark-adapted' when exposed only to green light (like rhodopsin rods in red light), to exhibit their sensitivity in the blue part of the spectrum and to be depressed by adaptation to blue light. All these expectations are satisfied by the receptors responsible for the blue hump of Figs. 5 and 6, and in addition the Stiles–Crawford evidence shows that the receptors involved are in fact not cones. We conclude that in the mesopic state pink rods, green rods and cones all contribute to retinal excitation.

PART II

The threshold during dark-adaptation

Since the pioneer experiments of Kohlrausch (1922) and their thorough analysis by Hecht and his colleagues (1937) it has been confirmed countless times that during the dark-adaptation which follows exposure to a bright light, the log. threshold for human vision falls upon a curve which in general exhibits a well marked kink. The first branch is due, at least mainly, to cones, for it is present, and present alone, when the test light falls within the fovea or is deep red in colour and so is without effect upon the rods. The second branch is due to rods, and rods only, for it is absent in the conditions just stated, the sensation produced by the test flash is the scotopic grey no matter what the test colour, and the spectral sensitivity corresponds to the absorption of rhodopsin. The transition from cone to rod is abrupt with a sharp kink in the curve and this strongly suggests that signals from cones and rods are independent, the threshold at any moment depending simply upon which is the more sensitive.

Now Granit (1942) has shown that a similar two-branched curve can be obtained from the frog's eye when threshold measurements are made during the course of dark-adaptation, taking as index of excitation the spikes from single ganglion cells or the restricted discharges from a small area. He pointed out that the first branch was determined by cones, since the spectral sensitivity here was maximal around 560 mμ (and in some cases even nearer 600 mμ). In the second branch the sensitivity maximum had shifted towards 500 mμ, so that presumably this was rod-dominated. However, the spectral sensitivity did not at once coincide with that of rhodopsin, and in the early stages a marked increase in blue sensitivity was noted. Only at a very late stage in this second phase of dark-adaptation was the pure rhodopsin sensitivity obtained.

We have repeated these experiments of Granit upon dark-adaptation, and directed particular attention to obtain the best possible isolation of single ganglion cells (cf. Barlow, 1953, for evidence concerning the single-cell nature of this discharge). Our results confirm Granit in all essentials. A preliminary report of this work has already appeared (Donner, 1958).

PROCEDURE AND RESULTS

The eyes were excised in daylight and were further light-adapted with the full white exposure from the apparatus. Then the adapting light was extinguished and measurements of absolute threshold were started. These were flashes of 1 sec duration and of intensity just sufficient to produce a discharge. As each threshold was determined, the time in the dark was noted. The test flashes were of six different colours and Fig. 7 shows the results of one experi-

ment where log. quantum threshold is plotted against time in the dark for each wave-length of the test. The curves have been given arbitrary vertical displacements so that they lie clear of each other in the figure. It is plain that all, except at the shortest wave-lengths, show a pronounced kink in the curve.

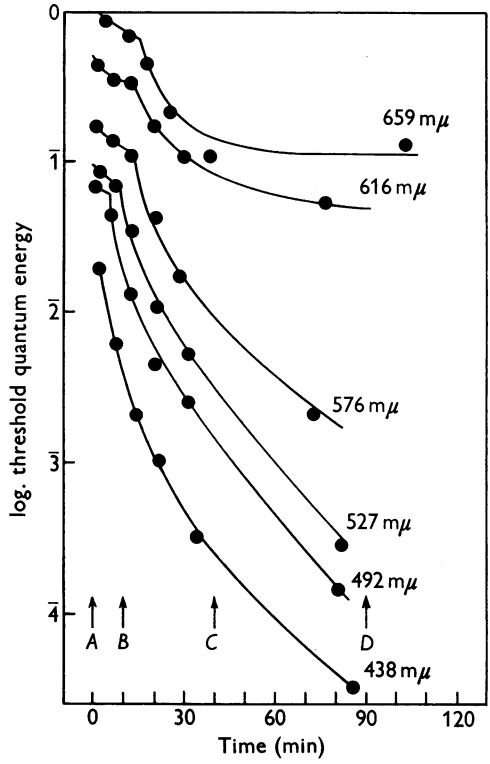

Fig. 7. Log. threshold dark-adaptation curves of single ganglion with test flashes of wave-lengths shown. The curves show two branches, photopic and mesopic. Curves displaced vertically for clearness.

The branch before the kink might be described by a single curve slid vertically, but certainly this cannot be said of the branch beyond the kink. This means that, unlike the human eye, this branch is not concerned simply with the change in sensitivity of the rhodopsin rods (or any constant mixture of receptors).

The spectral sensitivity curve at any time during the dark may be constructed from the curves of Fig. 7 (making allowance for the arbitrary displacement). It has been done for the times indicated by the arrows *A–D* (Fig. 7), and the resulting curves are plotted in Fig. 8 where the black circles represent points upon the first branch of the dark-adaptation curve, the white circles points upon the second. Continuous freehand curves join the points,

and for comparison the upper dotted curve is the absorption spectrum of rhodopsin, and the lower two are our redetermination of Granit's (1942) photopic dominator curve (Donner & Rushton, 1959, Fig. 4). We confirm Granit's conclusion that the first branch of the curve (black circles) corresponds to the photopic dominator, and that the second branch (white circles) exhibits a rapid rise of sensitivity in the blue and a general shift of sensitivity towards

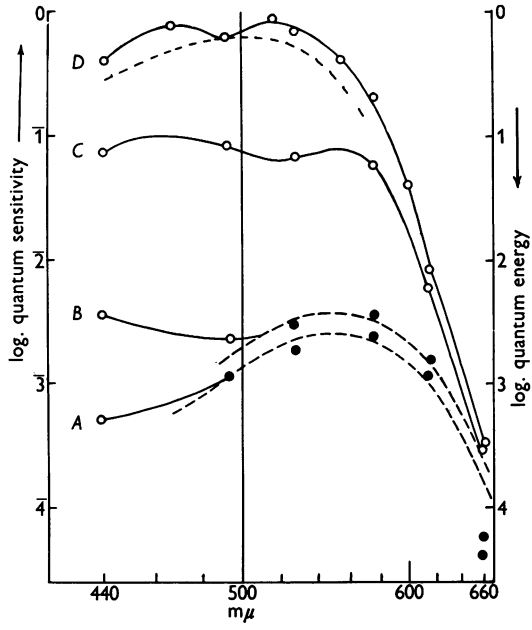

Fig. 8. Points of Fig. 7 replotted to give spectral sensitivity at times *A*, *B*, *C*, *D*. Points before the kink (Fig. 7) plotted black in Fig. 8. Upper dotted curve is rhodopsin; lower dotted curve is the photopic dominator: full curves drawn freehand through the points.

a maximum around 500 mμ. At 90 min of dark-adaptation some additional threshold points were added, so that curve *D* gives a clear picture of the spectral sensitivity at this time, which resembles closely the sensitivity analysed by the Stiles–Crawford effect in Fig. 5.

DISCUSSION

The dark-adaptation curves therefore support the results of silent substitution in defining three retinal states. The photopic state has the spectral sensitivity of the photopic dominator and is so stable that none of the procedures described has caused any detectable change in it. It could be simply explained by the hypothesis that there exists a single type of 'photopic cone' with the required spectral sensitivity, and in the photopic state no other photoreceptor

contributes appreciably to sensitivity. On the other hand, the frog's retina contains two types of cone which are histologically distinct (Rochon-Duvigneaud, 1943, Fig. 212; Saxén, 1954), and our results are consistent with the view that both types contribute in a fixed proportion to photopic excitability in the conditions studied.

The mesopic state generally involves cones, rhodopsin rods and grass-green rods in varying proportions, so there is a sharp distinction between the curves of Fig. 7 and the corresponding human results, where the second branch of the curve is of the same shape independent of test colour, and corresponds exactly to the spectral sensitivity of rhodopsin. In the frog, as may be seen from Fig. 8*B*, immediately after the photopic curve has been left there is a rapid increase of excitability in the blue which the Stiles–Crawford results show to be associated with the grass-green rods. The presence of the direction-sensitive cone hump in the green (Fig. 8*C*, *D*) shows that transition to the second branch of the adaptation curve does not mean (as it does in man) that cones are no longer involved. Both blue and green humps are supported upon the general sensitivity of the mass of rhodopsin rods in a way which will be analytically described later. At the moment the statement receives support from the fact that the green hump may be removed by oblique incidence of light (Fig. 5) and the blue hump by adaptation to blue radiation (Fig. 6) and in each case the underlying rhodopsin sensitivity is revealed. Since it is clear that three different and histologically distinct kinds of receptor contribute to mesopic sensitivity, a new and very interesting problem arises. It is easy to accept the conclusions of the present paper that the blue hump is due to the 'grass-green' rods and the green hump due to one or more types of cone, each contributing to the threshold in its own domain of the spectrum. But how then are we to explain 'silent substitution'?

The facts of the previous paper are now seen to be that when a retina has been adapted to a light which (say) excited mainly rods, we may always change to a new light exciting the cones more and the rods less in such a way that the recorded ganglion discharge does not detect the change. The condition for silent substitution is that the intensity must be exactly right, at least within a narrow margin. The size of this margin turns out to be the same (on a logarithmic plot) for changes between any wave-length and any other, and in particular for a change in intensity only, without colour alteration. The margin therefore is simply the Fechner fraction.

It thus appears that the various rods and cones add together their respective contributions towards ganglion discharges in some kind of 'excitation pool', and that the Fechner increment threshold depends simply upon the level of this pool, but not upon the way in which it is made up. This raises some interesting questions as to what is to be expected of increment threshold when a flash of one colour is superposed upon a background of another colour, and how far do

the results correspond with the psycho-physical experiments of Stiles (1939, 1949, 1959). The discussion of 'excitation pools' will thus be left until these increment threshold results have been described.

SUMMARY

1. The Stiles–Crawford retinal direction effect was examined in the excised eye of the frog with lens removed, by using as index single ganglion discharges.

2. As in man, the cones are sensitive to the direction of light incident upon the retina, but the rods are not.

3. The two humps of excitability in the mesopic spectral sensitivity curve were investigated in this way. The green hump is direction-sensitive and therefore involves cones: the blue hump is insensitive and hence does not.

4. The blue hump is depressed by strong blue light but not by strong green light. It is due therefore not to cones and not to rhodopsin rods but to the 'grass-green' rods which constitute some 10% of the frog's rod population, and which are blue-sensitive.

5. Dark-adaptation curves plotted in the usual way show a marked kink. Before the kink the sensitivity corresponds to the photopic dominator. After the kink the sensitivity corresponds to the mesopic state and involves, besides the rhodopsin rods, green rods and some cones.

Our thanks are due to the Medical Research Council for a grant for apparatus.

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