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SIGNALS FROM CONES

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SUMMARY

1. We have studied red and green cones by contrast flash inhibition and found them both to be very similar to rods in their response to flash energy, except that all light quantities must be some 100-fold greater in cones for the same effect.

2. Using the methods of a previous paper (A.R.T. *a*) where no backgrounds were employed, we plotted log test flash λ against log surround flash ϕ with criterion that λ should just be detected. The experiment was repeated with a 'windmill stop' interposed in the ϕ flash which reduced its area symmetrically to $\frac{1}{8}$. From these two curves it is possible to extract the relation between N, the inhibitory signal, and ϕ, the test flash. It is

$$N = \phi/(\phi+\sigma),$$

where σ, the semi-saturation constant, is about 4·5 log td sec.

3. Using the methods of (A.R.T. *b*) where backgrounds were studied, we measured the increment threshold for the surround flash ϕ against its background θ using as criterion not that ϕ should just be seen but that it should generate a fixed inhibitory signal N so that the fixed test flash λ could just be seen.

4. This increment threshold curve resembled the Aguilar & Stiles (1954) curve for rods, showed saturation and a complete symmetry about the 45° line through the point with co-ordinates ($\log \theta_{\mathrm{D}}$, $\log \sigma$).

5. These results imply that the cone signal N is related to flash ϕ and steady background θ by

$$N = \frac{\phi}{\phi+\sigma}\;\frac{\theta_{\mathrm{D}}}{\theta_{\mathrm{D}}+\theta},$$

where θ_{D} is receptor noise (= *eigengrau*), and ϕ and θ are expressed in units of quantum catch.

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6. The ordinary increment threshold for cones does not show saturation because a steady saturating background bleaches all the pigment away. When the background is presented for only 100 msec with dark pauses between, no great bleaching occurs and saturation is seen.

INTRODUCTION

In two recent papers (Alpern, Rushton & Torii, 1970*a*, *b*, which will be referred to as A.R.T. *a*, *b*) the technique of contrast flashes (Alpern, 1965) was used to measure the size of rod signal set up by a 100 msec flash. It was found that the signal was directly proportional to flash energy over a range of some 4 log units, above which level the signal began to saturate following the same saturation curve as electrical signals recorded intracellularly from receptors or S-potentials.

Such electrical signals are found from cones as well as from rods, so it is natural to suppose that our contrast flash technique might be equally applicable to the measurement of cone signals. However, the saturation that Aguilar & Stiles (1954) found in their study of the increment threshold in rods has not been seen in Stiles' very extensive investigation of cones. So there is some break-down in the rod–cone correspondence that needs examination.

METHODS

The equipment and general procedure were as described in the previous papers (A.R.T. *a*, *b*). Figure 1*a* shows the spatial arrangement of the fields presented. The test flash λ fell on the 20′ central area that was centrally fixated. The contrast flash ϕ was presented upon an 8° annular surround that had a 20′ black centre where λ fell. A steady background μ could be applied to the central area and a background θ to the surround. The time relations are shown in Fig. 1*b*.

In some experiments a $\frac{1}{8}$ 'windmill stop' was interposed (as in A.R.T. *a*, *b*). This consisted of a stop in the ϕ flash which blocked off the annulus except for the $11\frac{1}{4}$° sectors shown between the dashed lines of Fig. 1*a*. In this way the area of the surround flash was reduced to $\frac{1}{8}$ and the inhibitory signal N was reduced to $\frac{1}{8}$ of what it was with the stop removed. On account of the superior acuity of cones, some care was taken to fashion the stop accurately and place it in sharp focus.

The subject's head was clamped in position with bite-board and viewing hood as in previous experiments, and his pupil was dilated with 1 % Mydriacyl. Two small points of light (F.L.) served to orient the gaze, the observer fixating midway between them. They were seen by reflexion in a cover slip placed immediately in front of the Maxwellian view lens which focused the images of the aperture stops of the (ϕ, λ, μ, and θ) beam onto the eye pupil.

RESULTS

1. *Contrast flashes on dark background*

(*a*) *Green cones.* This experiment is a repetition on green cones of the rod experiment (described in A.R.T. *a*). As shown by Alpern & Rushton (1965), if the test flash λ excites green cones at threshold, then it is only the green cones excited by ϕ in the surround which contribute to the rise in λ threshold. Other cones and also rods are excited, but they are without effect on the λ threshold. Similarly, if red (or blue) cones are excited by λ at threshold, it is only the red (or blue) cones in the surround that contribute to the threshold rise. We used a white ϕ flash in order to obtain very bright flashes and green (500 nm) flashes for λ.

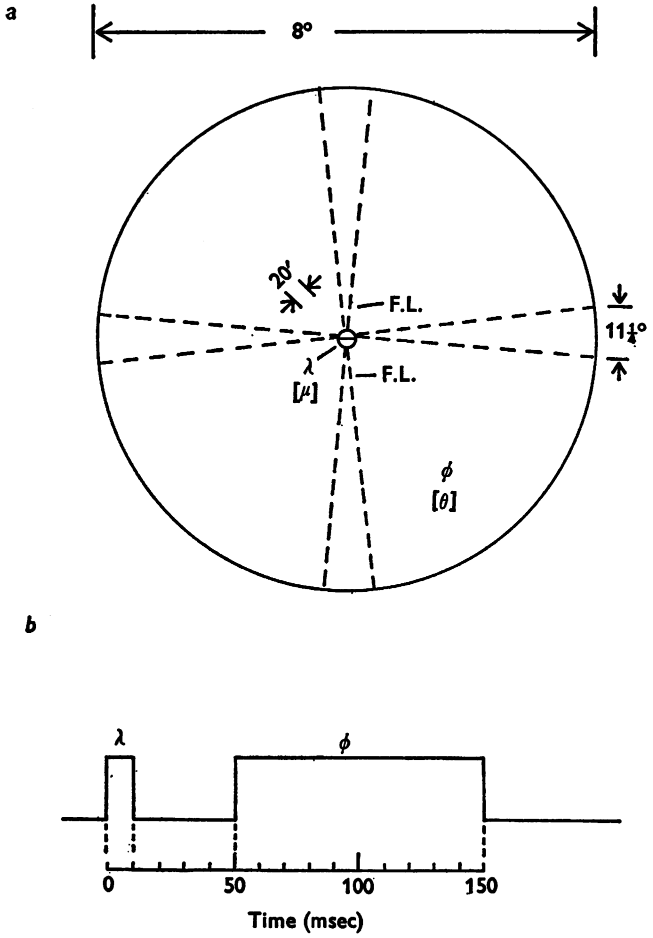

Fig. 1. (*a*) Spatial presentation of lights, fixated at centre of Figure. Test flash λ 20′ circle at centre; contrast flash ϕ on 8° annulus with 20′ black centre; steady background θ coincides with ϕ. 'Windmill stop' affects ϕ (but not θ) and reduces the surround to $\frac{1}{8}$ by permitting light to fall only on the $11\frac{1}{4}°$ windmill sails (shown between dashed lines). F.L. are two small orientation lights; the subject fixed between them.

(*b*) Time course of flashes: λ 10 msec, ϕ 100 msec duration; ϕ starts 50 msec after λ does.

Fig. 2 shows the result of one experiment where for each value of the surround flash ϕ presented, the test flash λ was adjusted so that it could just be detected. Log λ is plotted against log ϕ, both when the windmill stop was interposed (black circles) or not (white circles). The upper curve H_2 which plots the inhibitory signal N as log N against log ϕ is derived from the other two as Fig. 4*b* was derived from Fig. 4*a* in the former paper

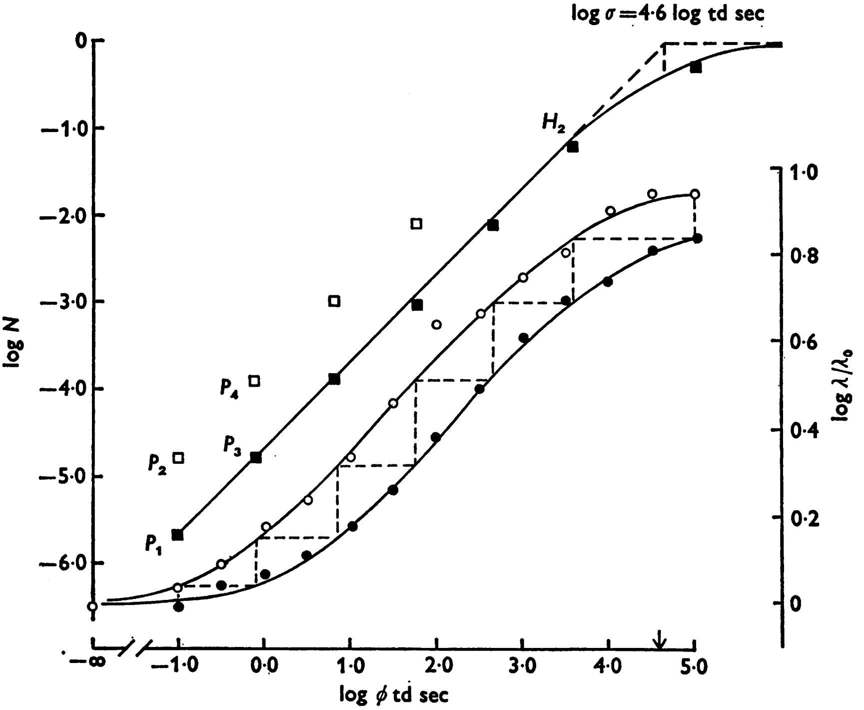

Fig. 2. White circles, log green (500 mm) test flash λ (scale on right) plotted against log (white) surround flash ϕ that just reduces λ to threshold. Black circles, the same but with windmill stop (Fig. 1*a*) interposed in ϕ beam. Smooth curves are drawn through the points; a dashed staircase is drawn between the curves, starting at the highest point. Black squares are placed above each dotted vertical and each square is 0·9 higher than the next. The curve H_2 drawn through the black squares is the theoretical H_2 curve that relates log N with log ϕ when $N = \phi/(\phi+\sigma)$.

(A.R.T. *a*). In Fig. 2 the lowest riser of the staircase is produced vertically upward to some arbitrary point P_1 marked by the black square that gives the log ($\frac{1}{8}N$) signal corresponding to the curve of black circles. Then the white square P_2 that marks the log N corresponding to the upper end of that riser must be 0·9 higher than P_1 since points on white circles differ from those on black circles below by the removal of the windmill and increase in N eightfold (or in log N by 0·9).

To obtain the black square P_3 which corresponds to the other end of the dotted tread, we note that the tread is horizontal, hence the λ value is unchanged along it; thus the inhibitory signal N must also remain unchanged. Consequently, P_3 must lie at the same level as P_2 and marks where that level meets the vertical extension of the second riser. P_4 lies above P_3 exactly 0·9 since the step corresponds again to the removal of the windmill stop.

Thus, the curve H_2 of log N against log ϕ is plotted by the black squares, each at a level 0·9 above the last and each at a distance to the right equal to the length of corresponding tread on the dotted staircase. It is seen that for most of its course H_2 is a straight line running up at 45°. This is because all the treads of the dashed staircase were 0·9 log units in extent. But at the top the treads are longer and so the line bends over. The curve drawn through this series of black squares is an H_2 curve (see Appendix to A.R.T. *a*), namely the relation

$$N = \phi/(\phi+\sigma) \tag{1}$$

plotted as log N against log ϕ. This is the same relation that describes the N signal for rods and also the electrophysiological responses recorded from receptors or as S-potentials. The value of ϕ, the semisaturation constant, is 4·6 log td sec, which is 2 log units above the σ for rods. The scale of log N on the left is shifted so that zero corresponds with the horizontal ceiling of the H_2 curve. The intersection of this with the 45° line marks log σ on the axis of log ϕ.

Red cones. In Fig. 3 the results were obtained using a red light (630 nm) for λ the test flash, and in consequence it was the red cone inhibitory signals from ϕ that were measured. The nature of the results and their analysis is precisely similar to those of Fig. 2. The value of σ is 3·6 log td sec, a log unit below σ for green. This is probably too low since other curves and other methods do not sustain it. The H_2 curve is derived from the dotted staircase as before, but the construction is omitted.

2. *Contrast flashes on bright backgrounds*

In our previous paper (A.R.T. *b*) we used also a second technique to demonstrate and measure saturation, a modification of that of Aguilar & Stiles (1954). They measured the increment threshold of rods against backgrounds in conditions where cones were not involved, and found that as log backgrounds became very strong, the log threshold increased even faster, so the curve rose above the Fechner 45° line and soon ran vertically signifying saturation.

We obtained the same curve (A.R.T. *b*, Fig. 8) using the contrast flash technique. In our case the flash that was projected onto a bright, steady background was not the test flash to be detected, but the contrast flash ϕ on its background θ (Fig. 1*a*) whose inhibitory signal N raised the threshold of the test flash λ. When λ was kept fixed, it was reduced to threshold by a fixed inhibition N; consequently our technique measures the relation between ϕ and θ for a fixed N.

Fig. 8 (A.R.T. *b*) showed a family of increment threshold curves each with a different fixed λ criterion. They all resemble the Aguilar & Stiles curve, which indeed is the lowest obtainable member of this family.

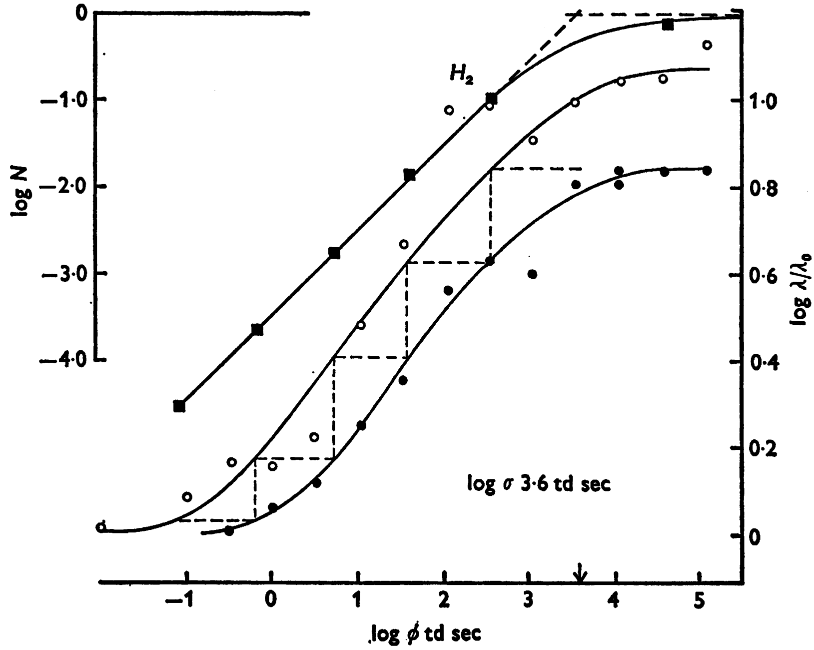

Fig. 3. Similar to Fig. 2 except that λ is here red (630 nm); thus red cones' signals are measured: ϕ is again white. The value of σ is probably too low.

It is to be noticed that the higher the curve, the lower is the background required for saturation. This gave us hope that we might get around the difficulty (whatever it was) that prevented Stiles from demonstrating saturation in cones (as he had with rods) since our contrast flash technique can bring on saturation at relatively low θ values.

Figs. 4 and 5 show that this hope is fulfilled and that saturation can be demonstrated in green and red cones by constant flash increment threshold. The measurements of Figs. 5 and 4 were obtained as follows. (*a*) For each background θ chosen, the threshold value of log λ_0 was found for the test when $\phi = 0$. This measures the effect of light scattered from θ. (*b*) At the level where log $\theta = 2{\cdot}6$, ϕ was made as strong as possible (5 log td sec) and log λ_1, the test threshold in this condition was read. This increase in log λ (0·47 in Fig. 4) is the criterion level for the whole curve. It is defined by the rectangular point at the summit of the curve. (*c*) At all other θ levels the value of log λ_0 found was increased by 0·47 (in Fig. 4) to reach the criterion, and ϕ was then adjusted so that this λ_1 value became just threshold. The log ϕ so obtained is the ordinate of Fig. 4, where the test flash was green, ϕ was white, and the steady background θ was also white.

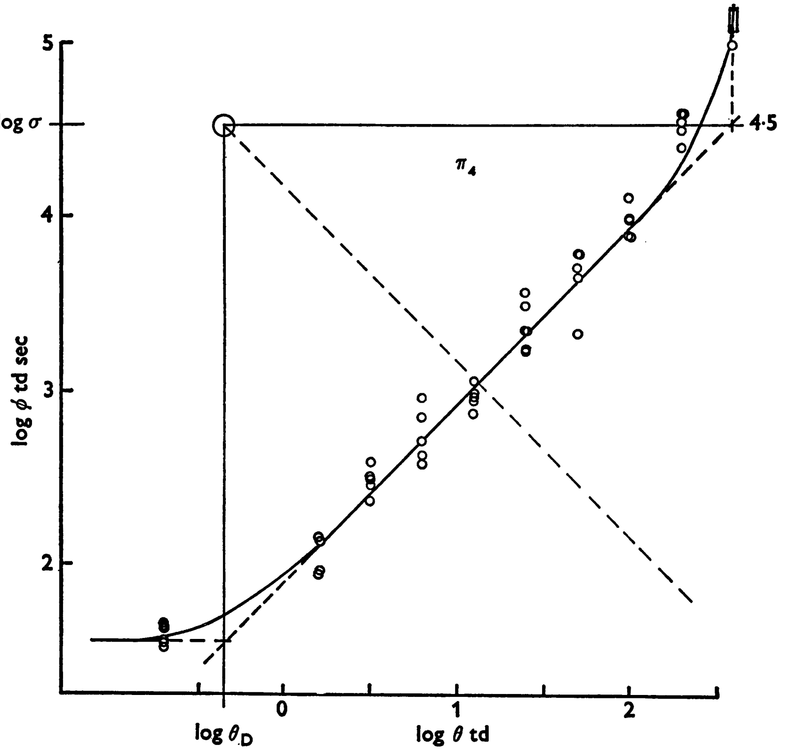

Fig. 4. Increment threshold curve for green cones plotted as log ϕ flash against log θ, the background on which ϕ falls, using as criterion that flash λ the fixed green test flash (log λ/λ_0 = 0·47) should always be reduced to threshold by ϕ. This is the criterion that ϕ shall generate a fixed signal N from green π_4 cones. The curve is symmetrical about the −45° line through O (co-ordinates: log θ_D, log σ). Cones saturate with a σ value of 4·5 log td sec, as in Fig. 2. ϕ and θ were white.

The increment threshold curve in the lower part corresponds to Fechner expectations, and in the upper part saturation sets in along a curve which mirrors the lower half in the −45° dashed line through the point (θ_D, σ). The value of σ is about 4·5 log td sec as in Expt. 1 of this paper. The experiment of Fig. 5 is like that of Fig. 4 except that now the λ flash was red; thus it was the red cones whose increment threshold and saturation were measured. The contrast flash increment threshold curve here also runs the Aguilar & Stiles' course and saturates with $\sigma = 4{\cdot}25$ log td sec, which is slightly below the green cone σ but much nearer to it than the 3·6 of Expt. 1.

We conclude that the cone signals are essentially the same as rod signals as a function of flash intensity, but with constants θ_D, and σ some 2 log units higher.

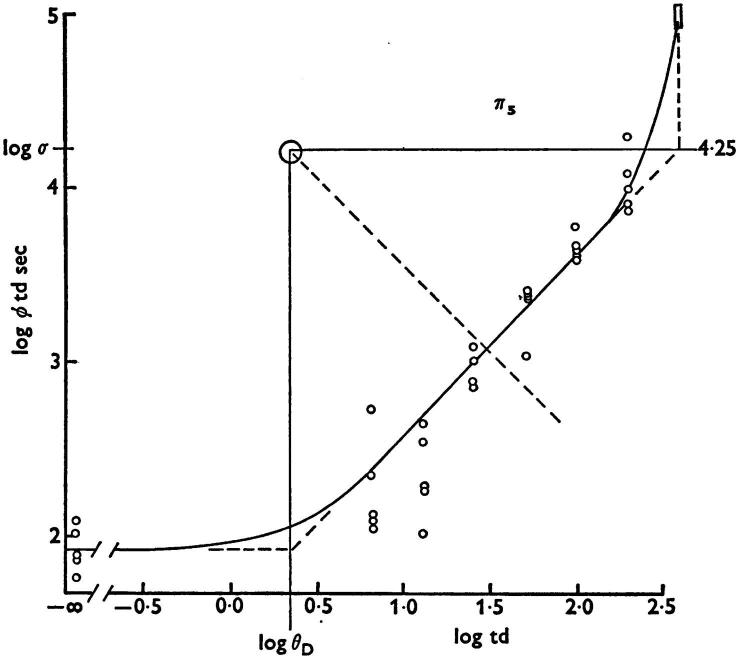

Fig. 5. Similar to Fig. 4, but λ is a red flash; thus, these measurements apply to red π_5 cones. The fixed test criterion ($\log \lambda/\lambda_0$) was 0·34. Here $\sigma = 4{\cdot}25$ log td sec, ϕ and θ were again white.

3. *Increment thresholds against brief backgrounds*

The presence of saturation in the curves of Figs. 4 and 5 poses the question 'Why was this saturation not seen in Stiles' (1939) careful and extensive increment threshold curves for cones?' Pigment bleaching by the steady background has often (erroneously) been proposed to explain the presence of saturation in rods; we propose it to explain the absence of saturation in cones.

Naturally the efficacy of any light stands in proportion to the number of quanta absorbed, and, if a large fraction of the visual pigment is bleached, the quantum catch will be proportionally reduced. In our previous papers (A.R.T. *a*, *b*) and in the present paper the reduction of the visual pigment by bleaching has been negligible, but this might not be the case when very strong steady backgrounds are applied and left on while an increment threshold curve for cones is obtained. From the known kinetic equations for cone pigments (Rushton, 1958, 1963; Rushton & Henry, 1968) the effect of backgrounds may be calculated

$$\frac{-t_0 \mathrm{d}p}{\mathrm{d}t} = \frac{\theta p}{\theta_0} - (1-p), \tag{2}$$

where t is time, t_0 is the time constant of regeneration ($=$ 120 sec), p the fraction of pigment unbleached, θ the steady background, and θ_0 the background level which at equilibrium bleaches 50 % ($\theta_0 = 4\cdot3$ log td). In equilibrium $\mathrm{d}p/\mathrm{d}t$ vanishes and we get (rearranging)

$$\frac{1}{\theta p} = \frac{1}{\theta} + \frac{1}{\theta_0}. \tag{3}$$

From eqn. (3) it follows that the effect of a background θ upon (θp) (the quantum catch) depends upon whether θ is less or greater than θ_0. If much less, (θp) is proportional to θ and we are justified in using θ itself to measure background efficacy. But if θ is large compared with θ_0, further increase in θ causes very little increase in (θp), and the quantum catch (θp) can never exceed θ_0, which is the catch that would result from a steady background of 4·3 log td if it still acted on unbleached pigment ($p = 1$).

Thus it is plain that if cones need for saturation a background catch that is greater than θ_0, no equilibrium background can saturate. Saturation, if obtainable by steady background, must be by one *not* in equilibrium – one that lasts long enough to raise the threshold but not enough for much bleaching, 100 msec for instance. This has been done in the experiment of Fig. 6.

In this experiment the background field was a uniform white 8° circle (with no black centre). The test flash was a white 1° circle concentric and centrally fixated. The background was presented for 100 msec and the test for 20 msec during the middle of the 100 msec background exposure. Fig. 6 shows the log threshold for λ, the test flash determined in this way against log background, the uniform $(\mu+\theta)$ of Fig. 1. The curve was obtained by a procedure similar to that of Aguilar & Stiles (1954) except that foveal cones were studied (not rods) and backgrounds were presented only in 100 msec flashes (instead of continuously) so that no substantial bleachings occurred. The curve shows the usual 45° Fechner line that rises to saturation at a background level of some 5 log td sec. The semisaturation σ of the combined cone mechanisms is the log λ where the Fechner 45° line meets the saturation vertical. This level is at $\sigma = 4\cdot7$ log td sec, which agrees with the two earlier estimates.

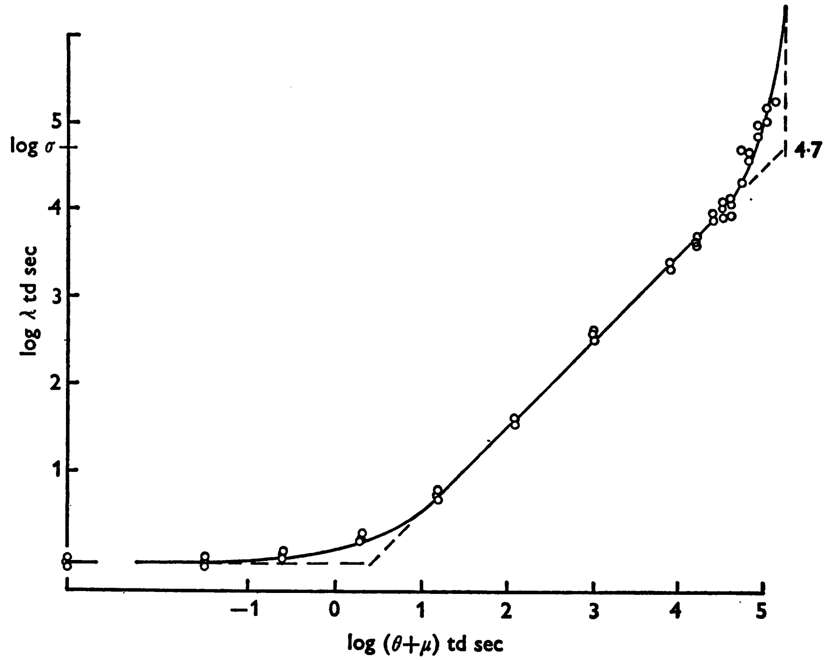

Fig. 6. Increment threshold for detecting white λ 1° flash against a white uniform background of 8°. The background was presented for 100 msec in the midst of which λ was flashed for 20 msec. In this way, bleaching was negligible and strong backgrounds produced saturation in cones just as Aguilar & Stiles (1954) had found in rods.

DISCUSSION

In this paper we have investigated cones by methods that have established for rods that the inhibitory signal N is related to ϕ the flash that elicits it and θ the steady background upon which ϕ falls (both expressed in units of quantum catch) by the expression

$$N = \frac{\phi}{\phi+\sigma}\,\frac{\theta_{\mathrm{D}}}{\theta_{\mathrm{D}}+\theta}. \tag{4}$$

We have found the methods satisfactory and the results very similar to those of rods. In Expt. 1, where no steady backgrounds were involved, we analysed the size of N by comparing effects with and without interposing the 'windmill stop' and found, as with rods (A.R.T. *a*, Fig. 4) that N was related to ϕ according to eqn. (4) with θ put zero. The black squares of Fig. 2 lie on the H_2 curve which is $N = \phi/(\phi+\sigma)$ when log N is plotted against log ϕ.

In Expt. 2 we examined the effects of background by determining the increment threshold curve against various steady backgrounds but using as criterion the strength of flash ϕ that will generate a signal N of fixed size, judged by its capacity to inhibit just to threshold the fixed test flash λ.

The results obtained were similar to those of Fig. 8 (A.R.T. *b*) and in particular the whole cone curve (Fig. 4) was also symmetrical about the $-45°$ dashed line that passed through the point O whose co-ordinates are ($\log \theta_{\mathrm{D}}$, $\log \sigma$). If we consider the curve plotted as $\log \phi$ versus $\log \theta$ referred to O as origin, the x-displacement will be $\log \theta - \log \theta_{\mathrm{D}} = \log (\theta/\theta_{\mathrm{D}})$; and the $-y$ displacement will be $\log \sigma - \log \phi = \log (\sigma/\phi)$.

Now eqn. (4) may be rewritten

$$\frac{1}{N} = \left(1+\frac{\sigma}{\phi}\right)\left(1+\frac{\theta}{\theta_{\mathrm{D}}}\right). \tag{5}$$

From this it is clear that if the values of σ/ϕ and $\theta/\theta_{\mathrm{D}}$ are interchanged it will make no difference to the size of N. Consequently, in Fig. 4, if the x and $-y$ co-ordinates of any point on the N curve are interchanged we shall get another point on the same N-curve. This means that an N-curve which satisfies eqn. (5) must be symmetrical about the $-45°$ line through O. The fact that our N-curve is found to be symmetrical is proof that eqn. (4) applies to cones as well as to rods. For the lower half of the curve, where σ/ϕ is very large, satifies Fechner's curve

$$N = \frac{\phi}{\sigma}\,\frac{\theta_{\mathrm{D}}}{\theta_{\mathrm{D}}+\theta},$$

which is certainly true, and only eqn. (4) gives the upper half with the observed symmetry to this lower half.

Alpern & Rushton (1965) showed that rods and the three types of cones each acted independently of each other in contrast flashes as Stiles (1939) had already shown for increment thresholds and Du Croz & Rushton (1966) for bleachings. In this paper we have investigated separately red cones and green cones, but blue cones which are relatively scarce and perhaps even absent from the central fovea (Willmer & Wright, 1945) have proved more troublesome. Stiles (1946) has shown that a blue light at threshold excites green cones, not blue; consequently to repeat the experiments of this paper and be sure that it is blue cones that are being measured requires more refinement than simply to use a blue λ flash. These experiments became rather troublesome and we have set them aside. We have so little reason to doubt that blue cones act like red and green cones that we have not persisted in the rather complex technique that is necessary to prove it.

Red and green cones are nearly identical in their response to light flashes and similar to rods except that θ_D and σ are both some 2 log units higher with cones. Perhaps σ for green cones is a little higher than it is for red cones. The failure to demonstrate cone saturation with steady background fields (as was done with rods by Aguilar & Stiles, 1954) is because, at the high background levels required, the cone pigments are bleached away. Obviously the physiological effect of light is produced not by the quanta incident on the cones but by the quanta absorbed, and, as the pigments become more bleached, the fraction absorbed falls. Thus, as log θ increases by equal steps, the log quantum catch, log (θp), increases by steps that start also equal but get smaller and indeed, with increasing rapidity, finally coming to a stop. As shown in eqn. (3) the greatest possible rate of quantum catch is θ_0, the rate when 4·3 log td falls upon unbleached cones. If this rate is below the saturating level, saturation by steady backgrounds will be impossible.

We may estimate this saturating background level from Fig. 4 where bleaching is negligible, and thus the axes may be regarded as plotting ϕ and θ in units of quantum catch. As we have seen from eqn. (5), plotted in this way the whole family of curves for different fixed N-values is symmetrical about the $-45°$ dashed line of Fig. 4. The curve whose saturation level we seek is the ϕ visibility curve, the curve where N corresponds to the visual threshold for seeing ϕ itself against θ. Now the threshold for seeing ϕ in the dark (the cone absolute threshold) is -1 log td (or less), and with a ϕ flash lasting 0·1 sec (as in Fig. 4) the ϕ visibility curve would level out at -2 log td sec, which is 3·5 log units below the level in Fig. 4. Thus, by $-45°$ symmetry, the ϕ visibility will saturate 3·5 log units to the right of the curve in Fig. 4. This is about 6 log td of quantum catch. It is thus plain that saturation was not to be expected with steady backgrounds since 4·3 log td is all the quantum catch they can give and about 6 is what is required.

We may circumvent inactivation of backgrounds through bleaching by presenting them for only 0·1 sec as in Fig. 6. Here the quantum catch will be proportional to the incident light up to very high values, and it is thus satisfactory to see that saturation occurs at the expected value of 6 log td, which, presented for 0·1 sec, is plotted as 5 log td sec.

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