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Saturation of a retinal cone mechanism

In discussions of the several differences between the rods and the cones of the human retina it is usually stated that rods saturate and cones do not. When measurements are made of the intensity (ΔI) of an incremental flash that can just be detected on a steady background field of intensity I, the cones normally continue to obey Weber's law ($\Delta I/I$ = constant) at very high intensities when nearly all their pigment has been bleached away; but when only a very small fraction of rhodopsin, the rod pigment, has been bleached the incremental threshold for rods begins to rise more rapidly than is predicted by Weber's law. This saturation of the rod signal can be observed when, by careful choice of wavelengths and by exploitation of the directional selectivity of the cones, the rod response is followed to high intensities in normal observers[1]; or when measurements are made on rod monochromats, whose retinae completely, or almost completely, lack cone receptors[2-4]. Alpern, Rushton and Torii[5] have suggested that it is bleaching that prevents saturation in the cones, for the receptor signal depends on the number of quanta actually absorbed and the latter quantity will tend to a limit as more and more pigment is bleached by steady background fields of increasing intensity. Cone mechanisms do saturate when intense, brief flashes are delivered to the unbleached retina[5-6]. We show here that the blue-sensitive receptors, which are normally thought to be cones, can be saturated by steady fields. In this respect they resemble the rods rather than the red- or green-sensitive cones.

Since the green cone mechanism is almost as sensitive to blue light as is the blue mechanism[7], it was necessary to choose experimental conditions carefully in order to follow the isolated response of the blue mechanism to high intensities. The test flash was violet (435 nm); and was large (1° of visual angle) and long (200 ms) in order to take advantage of the spatial and temporal integrative properties of the blue mechanism[8-9]. It was delivered to the fovea, fixation being guided by an array of four small fixation lights arranged in a diamond. A bright yellow (575-nm) 'auxiliary field'[10], which subtended 6.5°, served to maintain the light adaptation of the long- and middle-wavelength mechanisms; it produced a retinal illuminance of 5.48 log troland (equivalent to 0.17 log erg s^{-1} $degree^{-2}$) and remained present throughout the experiment. The primary adapting field (445 nm) was of variable intensity and was congruent with the auxiliary field. The arrangement of the stimuli is shown in Fig. 1*d*. The experiment was under computer control and a random double-staircase procedure[11] was used to measure the threshold for detecting the violet test flash at each of an increasing series of intensities of the 445-nm adapting field. Four minutes of light adaptation preceded measurements at each new level of the 445-nm field. Other details of procedure, apparatus and calibration were as described elsewhere[12].

Results are shown in Fig. 1*a* and *b*. The broken line has a slope of 1 and represents Weber's law. Clear evidence of the onset of saturation is seen in the rapidly rising increment-threshold function. Blue–violet fields that cause saturation are

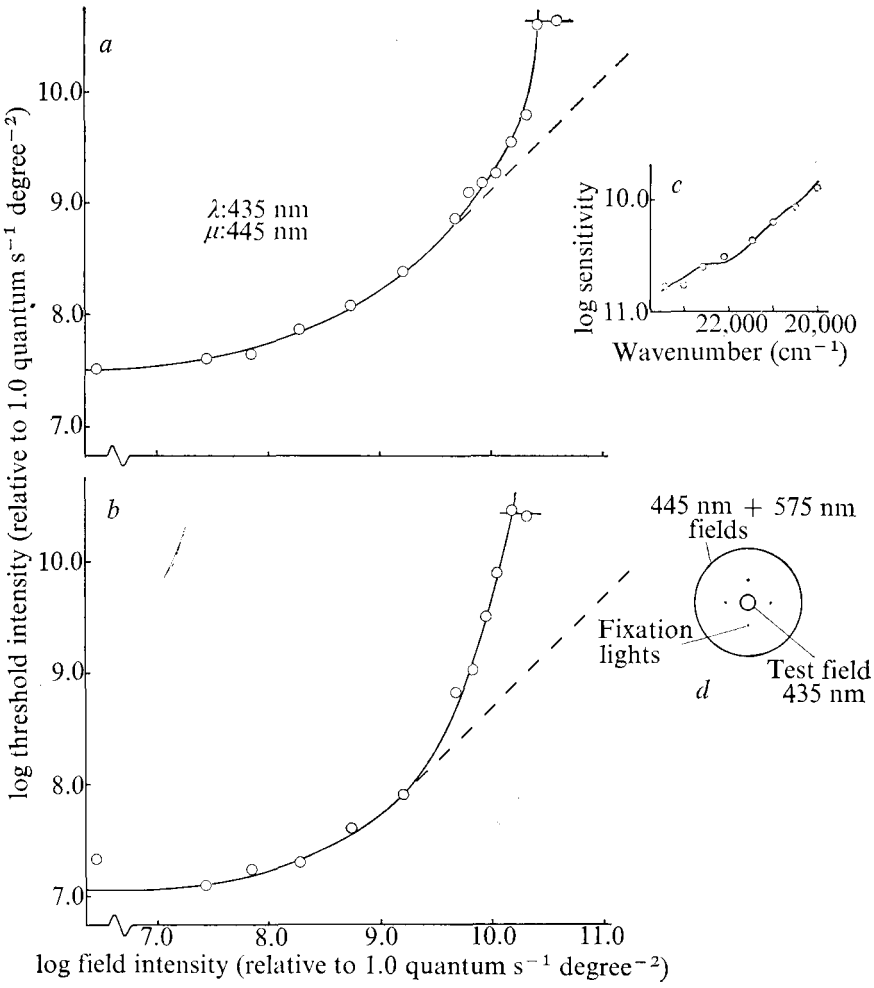

Fig. 1 *a*, *b*, Incremental threshold (ΔI) for the 435-nm test flash as a function of the 445-nm primary adapting field. The left-most point represents the threshold on the yellow auxiliary field alone. Observers: J.D.M. (*a*), P.G.P. (*b*). *c*, Spectral sensitivity of the eye after 4 min adaptation to the most intense 445-nm field (10.57 log quanta s^{-1} $degree^{-2}$). The ordinate is log sensitivity, that is, log reciprocal threshold (relative to 1.0 quantum s^{-1} $degree^{-2}$). The solid line represents the spectral sensitivity of Stiles's green mechanism, π_4, and has been arbitrarily displaced vertically. *d*, Arrangement of the stimuli.

not very bright: the increment-threshold function exceeds a slope of 1 when the 445-nm field has a value of ~ 100 trolands, although, of course, the photopic troland is not an appropriate measure of light absorbed by the short-wavelength receptors.

At the highest field intensities the function suddenly flattens, and we suggest that here the green cones have taken over the detection of the violet flashes. To test the latter proposal directly we measured the spectral sensitivity of the eye when it was adapted to the most intense 445-nm field, the auxiliary field still being present: different test wavelengths were used in random order and measurements were always made between 4 and 10 min after the onset of adaptation, during which period the threshold is known to be stable for the observer used (J.D.M.). Measurements were made at two wavelengths in each session and at least 3 h elapsed between sessions. The results (Fig. 1*c*) are well fitted by π_4, Stiles's green-sensitive cone mechanism[7]. They could in principle be fitted by the red mechanism, π_5, but definitely not by π_3, the blue mechanism (which is plotted in Fig. 2*c*).

The reader will ask why saturation of the blue receptors has not previously been recorded, either by Stiles or by the many others who have measured increment thresholds using his two-colour procedure. A simple explanation is that many experimenters have not had available the necessary intensities of short wavelength light. But since Stiles is usually cited in support of the claim that cones are never saturated by steady fields, it is interesting and reassuring to note that Fig. 11 of the paper[10] published by Stiles in 1953, which represents data collected in conditions similar to those used here, does show an increment threshold function with a final slope that is greater than 1. Stiles used a 555-nm auxiliary field of -2.15 log erg s^{-1} degree^{-2}, which was less intense than that of the present experiment: thus the intrusion of π_4 would occur earlier and would mask the most steeply rising part of the function of Fig. 1*a* and *b*. Norren and Padmos[19] have presented electroretinographic evidence that the dynamic range of the blue mechanism is limited.

What causes Weber's law to fail for the blue receptors? There is good evidence that at lower intensities the blue mechanism is subject to inhibition from a long-wavelength mechanism and that Stiles's three blue mechanisms, π_1, π_2, and π_3, correspond to the same receptors in different states of inhibition (for discussion see refs 12 and 13). Does the saturation seen in Fig. 1 arise directly from quanta absorbed by the short-wavelength receptors or is the blue mechanism being inhibited by a mechanism with a different spectral sensitivity? If the former is the case, we should find the lights of different wavelength that produce a given degree of saturation are lights that from other evidence would be thought to produce equal absorption in the blue receptors; but if the alternative hypothesis were correct, then the results of varying the wavelength of the adapting field ought to reflect the spectral sensitivity of the putative inhibitor. These expectations were the basis for a second experiment, in which the wavelength of the primary field was varied.

The conditions for the second experiment were similar to those of the first, but the wavelength of the auxiliary field (μ_A) was lengthened from 575 to 589 nm, in order better to reveal Stiles's π_3 mechanism, and its brightness was reduced to 5.2 log troland (-0.03 log erg s^{-1} degree^{-2}). The left-hand panel of Fig. 2 shows the effect of the orange auxiliary field alone on detection of the violet test flash. The three shallow branches correspond to Stile's mechanisms π_1, π_2, and π_3. The final level of the orange field (marked with an arrow) was that used as the auxiliary field during the main measurements and it is seen to raise the threshold by about one $\log_{10}$ unit.

Primary adapting fields of varying wavelengths were then

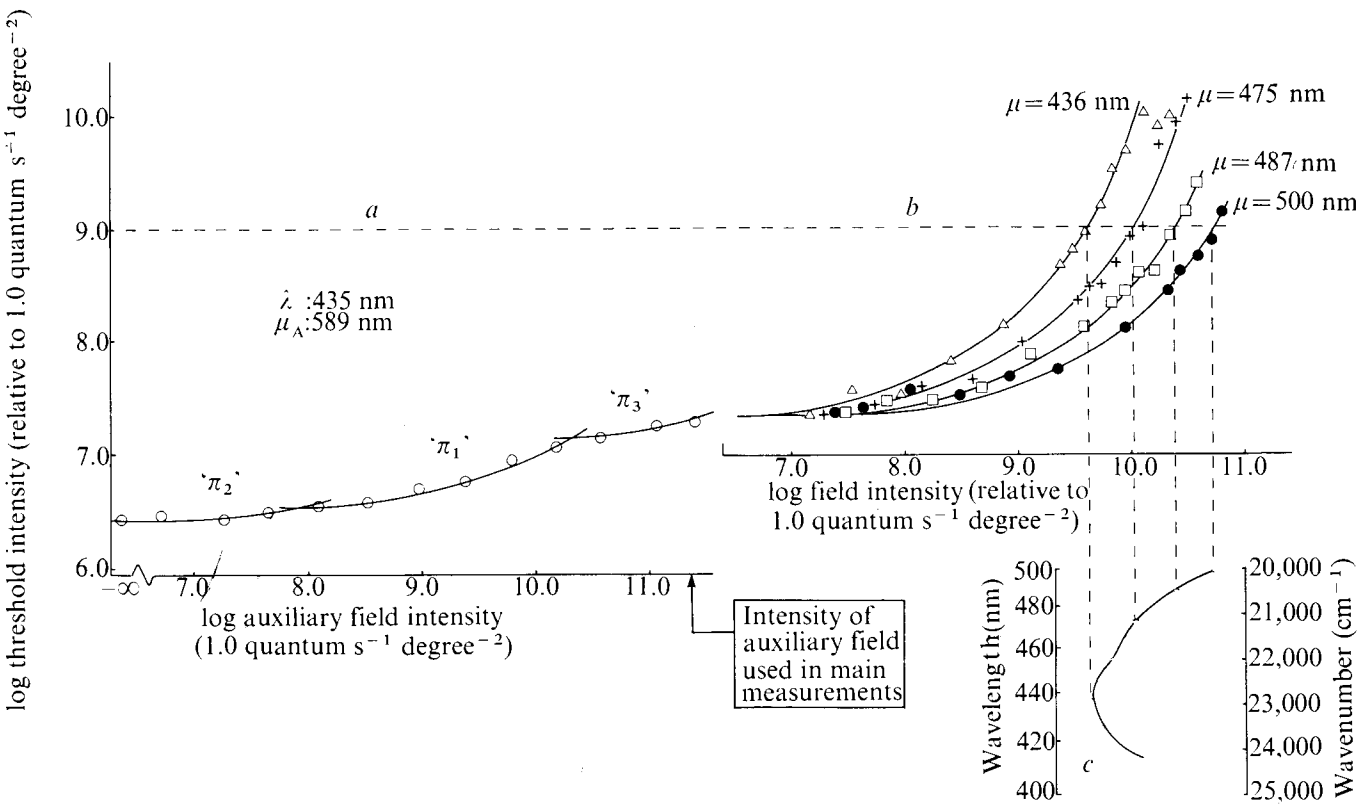

Fig. 2 *a*, Incremental threshold for a 435-nm test flash as a function of the intensity of the orange field alone. The curves fitted to the data points in each case correspond to Stiles's function $\zeta(x)$, taken from Table 7.5, ref. 7; and the relative position on the ordinate of the π_1 and π_2 branches is derived from the tabulated sensitivities of these mechanisms (Table 7.6, ref. 7). The reader should place less weight on the π_2 branch, which has been arbitrarily placed. Observer: J.D.M. *b*, Incremental threshold as a function of the intensity of the main adapting field. The parameter is the wavelength of the main adapting field (μ). The auxiliary field was present throughout. The solid curve is empirical and has been displaced laterally to give the best fit to each set of data. Note that quanta absorbed from the auxiliary field are not represented by the abscissa of this plot. (The theory of the auxiliary field is discussed by Stiles in ref. 10). *c*, Field intensities that produce criterion saturation plotted against wave number. The solid line represents the field sensitivity of Stiles's high-intensity blue mechanism, π_3.

added to the orange auxiliary field, a single experimental session being devoted to each wavelength. Results for four adapting wavelengths are shown in Fig. 2*c*; the results for other wavelengths are not shown to avoid congestion on the plot, but they are very similar in form.

Each set of data can be fitted well by a curve of fixed form that obeys Stiles's displacement rule[14], being displaced along the abscissa without vertical shift or other distortion. Figure 2 shows graphically how from these data can be derived the spectral sensitivity of the mechanism underlying saturation. We take as an arbitrary criterion that field intensity at which the slope of the increment-threshold function is 2. (Since the curve is of fixed shape, any criterion will give the same answer.) For each adapting wavelength this value is projected downwards and plotted against wavelength together with the corresponding points for sets of data not shown. The spectral sensitivity function that results (open triangles, Fig. 2*c*) is extremely well fitted by Stiles's curve for π_3, which is taken from ref. 7 (Table 7.6) and which can on several grounds be regarded as a good estimate of the spectral sensitivity of the blue receptors. That the fit is good must follow from the fact that the increment threshold functions of Fig. 2*b* have a fixed shape, for Stiles's measurements of the field sensitivity of π_3 were made in similar conditions but with a lower criterion.

Basic to this derivation is the assumption that Rushton[15] has called the principle of univariance: the output of a receptor depends only on the number, and not on the wavelength, of the quanta caught. As we vary wavelength, all that will change is the proportion of incident quanta that are absorbed. If the data of Fig. 2*b* could be plotted in terms of quanta actually absorbed then all the curves would coincide; but the abscissa is in fact the number of quanta delivered to the cornea and thus each curve is displaced laterally by a distance equal to the log of the ratio of quanta incident to quanta absorbed. It is these displacements that give us the spectral sensitivity of that which causes the saturation.

We conclude that the blue mechanism saturates when the blue-sensitive receptors are themselves absorbing a fixed number of quanta from a field. The present experiment offers no evidence for inhibition.

The saturation of the blue receptors at relatively low intensities may account for several long-standing problems of colour vision. It may, for example, explain why violet lights show a Bezold–Brücke hue shift towards longer wavelengths, coming to appear pale blue as their intensity is increased[16-17].

We have some evidence that if adaptation is maintained at saturating levels for more than 15 min, a recovery of sensitivity occurs: this further anomaly, which recalls physiological findings for rods in the skate[18], is under investigation.

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