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STILES–CRAWFORD EFFECT AND THE BLEACHING OF CONE PIGMENTS

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SUMMARY

1. The efficiency of light entering the eye through various points in the pupil (Stiles–Crawford effect) was studied using two criteria: (*a*) visual brightness judged by flicker fusion and (*b*) the rate of cone pigment bleaching measured by reflexion densitometry.

2. Both measurements were made in the same apparatus with the same geometry of presentation and both gave the same Stiles–Crawford effect.

3. This suggests that the densitometer measures pigment deep in the outer segments of the cones where light is absorbed for vision.

4. Foveal cones seem all to point in the same direction, since the fraction of pigment bleached by light entering the pupil at any one point is the same when measured by light entering anywhere.

INTRODUCTION

Stiles & Crawford (1933) discovered that foveal cones were excited more strongly when the light incident upon them entered through the centre of the pupil rather than through the periphery. In a careful study of the relation between the point of entry and the relative luminous efficiency Stiles (1937) found that there was a point of optimal entry. A field of luminance I_0 seen by rays entering here was compared with an adjacent field I seen by rays entering ρ mm away. When I was adjusted so that the two fields matched, it was found that

$$\log(I/I_0) = a\rho^2, \tag{1}$$

where a is a constant of about 0·05 per mm^2. This phenomenon has always been interpreted on the lines that rays of peripheral entry get less completely absorbed by the visual pigment in the cones. And since in the parafoveal retina the Stiles–Crawford effect applies to cones but not to neighbouring rods, the absorption failure cannot be due to light loss in the media

which rods and cones have in common, it must be loss within the cones themselves. Most workers accept some variant upon Toraldo di Francia's (1949) concept that the outer cone segment may be regarded as a dipole antenna, whose directional receiving properties account for the Stiles parabola.

We are not here primarily concerned to probe the details of this scheme but merely to test one very simple conclusion. If the Stiles–Crawford effect represents the efficiency of light absorption it should represent equally the efficiency for the bleaching of cone pigments as a function of the point of pupil entry. In fact we should find here (as everywhere) that lights appropriately matched for vision should be matched for pigment bleaching, objectively measured.

It might be argued that this correspondence is so obvious and inevitable that if it were not found one would suspect the experiments, e.g. that the densitometer was not measuring the pigment in the right place in the cone. Quite so; it is precisely this about which we should like to have some satisfaction.

If Brindley (1955) is correct that 0·98 is the density of erythrolabe in human cones, then 99% of the light will be absorbed on double passage if the light is reflected back through the pigment from the full depth of the outer segment. All work with reflexion densitometry indicates double densities much less than this, and in 1957 Rushton reported (1958*a*) 'These results then do not support Brindley's high cone densities, but neither do they refute them. For my catch may be from the shallows of the pigment pool, while he traps quanta in the depths.' If indeed quanta are being caught deep for vision and shallow for densitometry, we should certainly not expect the Stiles–Crawford attenuation with obliquity to act similarly upon them. But in fact the Stiles–Crawford effect appears identical in the two situations, and this seems strong evidence that the two situations themselves are identical.

The investigation we shall describe and the results we have found have already been treated by Ripps & Weale (1964). Their objective, however, was not primarily to investigate this matter (they tell us), and their experiments were not essentially designed to this end. We are glad to be able to confirm their conclusions by a technique that is very different from theirs, which possesses some advantages and covers a more extensive range of energies and of entry points.

Principle of experiment

(*a*) *Densitometry.* Our Florida densitometer (Hood & Rushton, 1971) is capable of bleaching and at the same time measuring the pigment level in human rods and cones. The bleaching light focused on the cornea formed

a 1 mm spot which could be moved vertically across the pupil. For each entry-point the intensity was adjusted so that the pigment level at equilibrium was (say) at 40 % bleached. Now for all occasions when the level bleached is 40 %, the rate of pigment regeneration is the same, and, since the pigment level was at equilibrium, the bleaching rate must also have been the same. Consequently at each point of pupil entry the intensity required for fixed equilibrium was the intensity required for a fixed rate of bleaching. The experiment was then repeated at the level of 60 % bleached. Naturally it needed more light to bleach down from 40 to 60 %; it was found that all lights had to be increased in the same proportion.

(*b*) *Psycho-physics.* The relation of brightness to pupil entry was measured with the same equipment using a neat application suggested by Dr M. Alpern. The bleaching light was reduced greatly in intensity but presented as before by the beam which entered the eye at a point that could be moved vertically across the pupil. The light was interrupted at 30 flashes/sec by a rotating vane and for each entry point the intensity was adjusted to attain critical fusion at this frequency. Since the C.F.F. curve that plots log intensity against frequency still rises steeply at 30/sec, our criterion of C.F.F. at 30/sec for each entry point is equivalent to a criterion of equal brightness. It was found that log intensity plotted against point of entry gave the well known Stiles parabola (eqn. (1)) and coincided with the same relation measured by densitometry.

METHODS

In principle the experiment has just been described; in detail it is as follows.

(*a*) *Densitometer.* The subject with pupil dilated with 1 % mydriacyl was aligned in the Florida densitometer and steadied by a dental impression and a brow rest. The bleaching light was focused onto a pin-hole that in turn was focused on to the cornea forming a 1 mm bright image. The beam passed through a plate of glass 1 cm in thickness that could be tilted about a horizontal axis and in so doing displaced the pupil entry point vertically without displacing the retinal image (except for a little spherical aberration of the eye). A pointer and calibrated scale attached to the mount of the glass plate permitted the entry point to be set immediately to better than 0·1 mm displacement from the zero point. The zero entry point coincided with the entry of the measuring beam of the densitometer and the subject was aligned so that this lay on the horizontal meridian of the eye slightly to the temporal side of the mid line so that no corneal reflex contaminated the signal returning from the fundus through the nasal half of the pupil. When the bleaching beam was sufficiently deviated it could be seen striking the iris at either extremity of the traverse. The deviation which just failed to do this was noted, and the experimental entry points kept within this range. The densitometer was set and used in routine fashion as described in Hood & Rushton (1971).

(*b*) *Flicker.* The subject was fixed in the same apparatus and in the same position and the flickering light was the same as the former bleaching light and entered through the tilted glass in the same way. Thus the bleaching light and the flickering light whose efficacies at various entry points are to be compared were in fact the

same light applied along the same paths. There were only two differences: (i) the vanes interrupted the light in both experiments but gave 10/sec flicker for pigment and 30/sec (which is more suitable) for cone C.F.F., (ii) the intensity level suitable for 40 % bleaching is far higher than that required for C.F.F. at 30/sec, so that the psycho-physical intensity levels were all much lower than those for bleaching. All lights used were white. Erythrolabe and chlorolabe regenerate at the same rate, and appear to bleach at the same rate with white light. Thus in our experiment we may treat the results as though only one cone pigment was involved.

RESULTS

Flicker. Measurements were taken at 0·5 mm intervals from 4·5 mm above to 4·0 mm below the optimum entry point of the pupil, settings being presented in haphazard order. For each point the subject made her own intensity settings by adjusting a wedge in the flickering beam first so that flicker just disappeared and then increasing until it just reappeared. This level was then recorded by the experimenter. A total of 270 settings were taken in eleven sessions, shown in Fig. 1 by the vertical lines which represent the 95 % confidence level (mean $\pm$ 1·96 S.E.). The curve is a parabola fitting eqn. (1) with 0·045 for the value of a. The good fit with Stiles's parabola is satisfactory but is no more than what many have confirmed before. It forms, however, a crisp curve against which we may compare the densitometry results with precisely the same light presentation.

Densitometry. In order to stabilize bleaching at 40 % (or any other fixed level) we must know the full range of densitometer wedge readings by finding the setting in darkness and at full bleach. But the pin-hole admits too little light for complete bleaching; therefore a preliminary full bleach reading was taken with the pin-hole removed. Then the pin-hole was restored and the greatest bleach now obtainable was found to be 80 % of all the pigment present. Knowing this, the wedge setting for 40 % or any other level can be made. It will be noted that *any* fixed level near 50 % will suffice for our measurement, for we are only concerned that lights at different entry points be adjusted to produce the *same* bleaching; we are not much concerned what that steady bleaching is. The main experiment now follows.

From preliminary measurements the intensity was known which bleached 40 % at central pupil entry (ρ_0). This light was applied and left on for 3 min to reach equilibrium and the exact wedge balance reading was taken. Then the entry point was changed haphazardly to a new place ρ_1 (e.g. -2 mm, Fig. 1) and simultaneously the log bleaching light was increased by the change in ordinate between ρ_0 and ρ_1, Fig. 1 (0·17 at -2 mm). Though it takes 3 min to reach the new equilibrium, the change is half complete in about 40 sec, so it was not hard to observe whether the new settings caused any change in the 40 % equilibrium level, and if so to trim

the intensity to restore it. In fact very little trimming proved necessary, and since the standard error of these % pigment readings was about $\pm 2{\cdot}5\,\%$ (of total pigment) we may conclude that very little change in equilibrium resulted from our change of entry when intensity was also changed according to the flicker results.

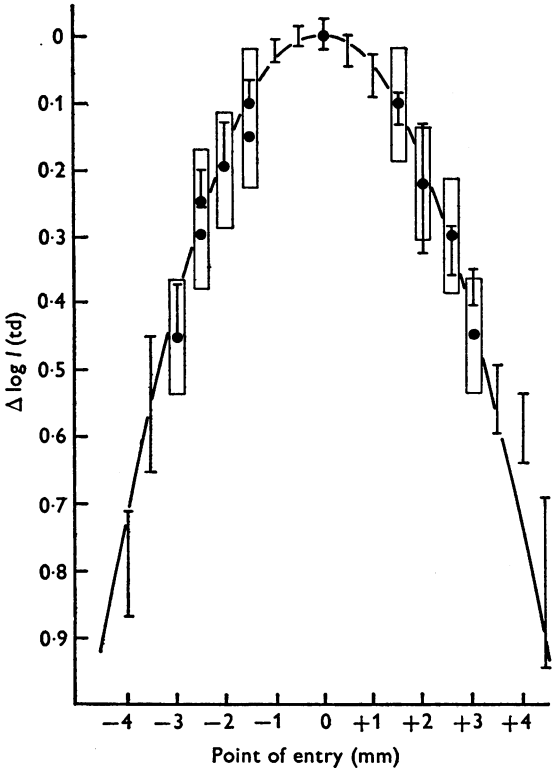

Fig. 1. Stiles–Crawford effect plotted as log efficiency of light against point of pupil entry in mm (ρ) from optimum place. The curve is the parabola $\log I = 0{\cdot}045\rho^2$. Lines show 95 % confidence level for equal brightness, as judged by constant flicker fusion. Black circles and rectangles show mean and 95 % confidence for equal bleaching rates of cone pigments (relative to that at zero entry). These were measured in equilibrium at the 40 and 60 % bleaching level; the two results coincide except at points $-1{\cdot}5$ and $-2{\cdot}5$.

In similar fashion the entry point was then changed haphazardly from ρ_1 to some other place ρ_2 and log bleaching light was simultaneously changed by the ordinate difference in Fig. 1 between abscissae ρ_1 and ρ_2. The whole series was then repeated using 60 % instead of 40 % bleaching as criterion.

The black circles in Fig. 1 show the average log intensities of bleaching

light, for various entry points each plotted relative to the value at central entry. Results with the 40% and 60% criteria so plotted coincide except at $-1{\cdot}5$ and $-2{\cdot}5$ where the two values are shown; elsewhere they are both represented by a single black circle. The rectangles enclosing the points represent in height the 95% confidence level of the observations estimated from the following considerations.

(*a*) The S.E. of all the measurements at each entry point were computed. They varied irregularly from point to point, and instead of plotting each in Fig. 1 we have taken the average of the S.E. and used that value to plot the rectangles for each point.

(*b*) Our measurements are % pigment present and our S.E. average is $\pm 2{\cdot}44\%$. We need to know what variation in log bleaching light gives this variation in % pigment. We may obtain this from kinetic theory or more directly from measuring the curves of Fig. 2 that plot the % level bleached at equilibrium against the logarithm of the bleaching light. At the 40–60% level the slope of the curve is nearly constant at 57% per log intensity, which is the same as 2·44% per 0·0044 log unit.

Thus the S.E. of $\pm 2{\cdot}44\%$ corresponds to a fluctuation of $\pm 0{\cdot}044$ log units of bleaching light, and the 95% confidence level (= 1·96 S.E.) is $\pm 0{\cdot}0855$ which is represented by the rectangles in Fig. 1.

Densitometry: a confirmatory experiment. We have been able to confirm the densitometry results by a different procedure whose results are shown in Fig. 2. As before, the known bleaching light enters at a determined point in the pupil and bleaches to measured equilibrium, but this time a whole curve relating the log intensity to the fraction bleached is obtained at one entry point. For instance, the filled circles of Fig. 2 show the results when the entry point remains central, and for the various $\log I$ values shown as abscissae the percent equilibrium bleachings are plotted as ordinates. Triangles, squares and open circles show the same thing when the entry point is 1·5, 2·5, 3·0 mm respectively below centre. It is clear that in general the more peripheral the entry the smaller the equilibrium bleach at fixed intensity; the stronger must be the intensity for fixed bleach. But the results show more than this. The curves are all one theoretical curve shifted in each case to the right of that through filled circles by a $\log I$ distance exactly equal to the ordinate difference of corresponding entry points in Fig. 1.

The kinetics of bleaching and regeneration are given by Rushton's equation (Rushton, 1958*b*; Rushton & Henry, 1968)

$$\frac{-t_0 \mathrm{d}p}{\mathrm{d}t} = \frac{Ip}{I_0} - (1-p), \tag{2}$$

where p is the fraction of pigment present, I the bleaching light, I_0 the

value of I which at equilibrium bleached p down to $\frac{1}{2}$ and t_0 is the time constant of regeneration. In equilibrium

$$I/I_0 = (1-p)/p. \tag{3}$$

This is the formula of the theoretical curve drawn through the filled circles in Fig. 2. It is drawn on a fixed theoretical template, which runs from zero to 100 % bleach, and we have to scale our experimental measurements to match it. We know that at central entry the full light from the pinhole

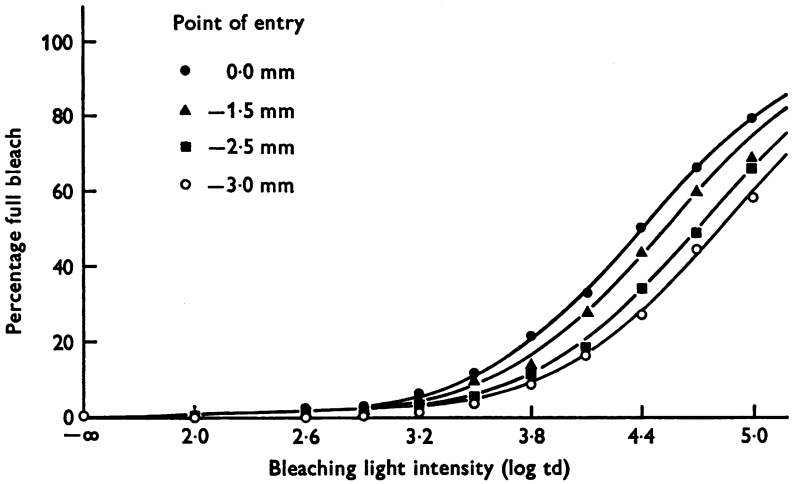

Fig. 2. Results of bleaching the cone pigments to equilibrium by lights of various strengths (abscissae). Bleaching light entered through optimum place near centre of pupil (filled circles) and at 1·5, 2·5, 3 mm below it (triangles, squares, open circles). The curve through filled circles is the theoretical curve from the kinetics of cone bleaching with 50 % bleach at 4·4 log td. The other curves are shifted to the right by the corresponding change in ordinate measured on the parabola of Fig. 1.

bleaches 80 %, thus all the density readings of Fig. 2 are scaled so that the top circle reads 80 %. The template is now slid horizontally to the place of best fit, which (as usual) is about $I_0 = 4{\cdot}4$ log td.

If light entering at ρ is less efficient for bleaching according to the curve of Fig. 1, then at $-3{\cdot}0$ mm entry lights must be 0·40 log td stronger than at central entry to do the same thing, and the template in Fig. 2 must be slid 0·40 log units to the right. The three template shifts in Fig. 2 were made according to these expectations from Fig. 1 and they are seen to fit the points reasonably well. The good correspondence strengthens the view that the Stiles–Crawford effect is the same for vision as for the bleaching of cone pigments that we measure.

Cone bouquets

It has generally been held that, in a cluster, all foveal cones point in the same direction, so the obliquity of light incidence is the same for them all. If, however, members of a cluster pointed in various directions like flowers in a bouquet, then part of the Stiles–Crawford effect would result from this heterogeneity of direction. Indeed, if it were possible for cones to be extremely restricted in their individual acceptance-angle, the cluster might still show the observed Stiles–Crawford spread simply due to the spread of the bouquet, as Safir & Hyams have recently pointed out (1969).

If light fell upon foveal cones, now by upper pupil entry now by lower, and if different cones in the cluster were chiefly excited in the two cases, it is not clear what difference in *sensation* (if any) would be expected. So the fact that little or no difference is generally observed is no disproof of the bouquet concept. But when bleaching rather than sensation is examined, expectation is clear.

If cones point in various directions and can only catch quanta by light arriving along their axis, then a bleaching light entering near the top of the pupil will bleach mainly cones pointing there. Also the measuring light, unless axial to a cone, will not be absorbed by its pigment, and hence cannot measure it. Thus to measure the bleaching in the present instance the measuring light also must enter near the top of the pupil, for rays from below can only measure cones pointing down, which are those which were not bleached by the light from above.

On this view then, we must expect great differences by pairs in the following four results; bleach from above or bleach from below, and in each case measure from above or from below. But if all cones point nearly in the same direction there should be no marked difference between any of the four results. This is what we have found to be the case.

Experiment and results

The experiment was performed like that of Fig. 2, except that the entry points for bleaching were only $+3$ and -3 mm, i.e. above and below the pupil centre to this extent. The measuring light also, instead of being the whole image of a vertical tungsten spiral near the pupil centre, was a short part of this filament (about 1 mm) entering either at -3 or $+1{\cdot}5$ mm from the centre.

Fig. 3 shows the four sets of results plotted as in Fig. 2, and the inset gives the key to the symbols, viz. circles when bleaching enters from below, white when the measuring enters from below. The points are each the average of four runs on two different subjects.

According to the extreme form of the bouquet concept, the only curves

which could show bleaching are when both lights come from below (white circles), or both from above (black squares); black circles and white squares should hug the base line. What is found, however, is that blacks run together and whites together, and the two curves are related better by a vertical scaling than by the horizontal shift of Fig. 2. This means that whether bleached from above or from below the pigment loss appears the same no matter from which entry point it is measured. So the direction of the bleaching light does not affect the amount of pigment bleached in any of the cones. It would be expected that black points in Fig. 3 should lie

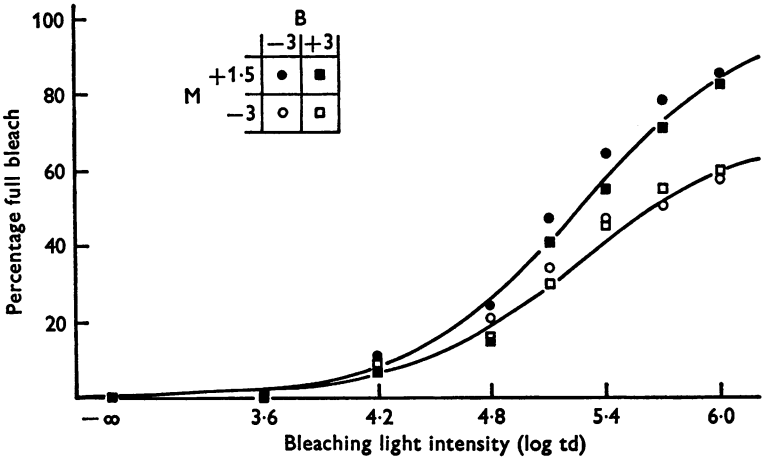

Fig. 3. Equilibrium bleaching by lights of various strengths plotted as in Fig. 2. Circles, bleaching light enters 3 mm below pupil centre; squares, 3 mm above it (see key inset). Black symbols, measuring light enters 1·5 mm above centre; white, 3 mm below. Ordinate scale and theoretical curve applies to black points; lower curve has ordinates 70 % of upper.

above the white, for in Fig. 2 the entry points 1·5 and 3·0 are represented by triangles and open circles whose curves run 0·3 log units apart. Thus light entering at 1·5 mm will be twice as much absorbed as that entering at 3 mm (log 2 = 0·3). Consequently in Fig. 3 at each bleaching level black symbols should record a density twice that of white (ignoring stray light and supposing that reflected rays retrace their ingoing paths. Perhaps a factor of 1·5 not 2 is more realistic).

We have considered the point of entry of light through the pupil; the point of exit now needs attention. Light that enters anywhere and illuminates the fovea is scattered back more diffusely and the 'red reflex' of ophthalmology fills the pupil; so light leaves everywhere. The emergent light, however, is about 10^{-4} of that entering and some 99 % is probably absorbed in the black pigment epithelium after once traversing the cones.

What then goes to other cones must produce quite negligible bleaching there. For the purpose of the present argument, bleaching may be considered as caused entirely by the in-going rays as treated above.

With measuring lights the matter is different. The light received upon the photocell has been down to the *fundus* and back and measures all the transmission changes encountered. If this light entered the pupil below centre and left above it (on the bouquet theory), it would record the pigment change equally of cones pointing down and of cones pointing up. In Fig. 3 the coincidence of white circles and white squares represents this condition where either the upwards-pointing cones (circles) or the downwards pointing (squares) are bleached. When we concluded above that the coincidence meant that cones did *not* point some up and some down, the fact that returning rays can also measure density was ignored – properly so; for hardly any such rays exist! On the bouquet concept the measuring rays entering pass all in one direction, and either all pass through bleached cones (white circles) or none pass through them (white squares). The returning rays go in all directions and only very few go through the bleached cones, simply those which leave the pupil by the bleaching entry point. And these, of course, go back to the bleaching light, not to the photocell. So they cannot contribute to pigment measurement.

DISCUSSION

If the Stiles–Crawford effect depended much upon a scatter of cone orientation, then light from one direction would bleach chiefly cones pointing that way. But the fraction of total pigment bleached is found to be the same when measured by lights from any direction, hence all cones act as though parallel. This does not mean that they *are* parallel, but only that the scatter in orientation is small enough compared with the cone acceptance angle for the Stiles–Crawford effect to be due principally to the latter. Indeed, the remarkable observations of Makous (1968) are most readily interpreted by supposing some non-parallelism within the cone cluster. He measured the increment threshold for a flash of fixed direction, presented upon a steady background which entered the pupil either (A) from one side or (B) from the other. If A and B were adjusted each to raise the flash threshold equally in steady conditions, a sudden switch from A to B was found to cause a large transient threshold rise, and later a sudden switch back caused a similar threshold rise. One asks, 'How did the cones "know" that the background had been switched?' The simplest answer is that not all the cones pointed the same way. The degree of non-parallelism sufficient to produce this sensitive Makous transient may well be insignificant in the steady-state measurements of the Stiles–Crawford

effect whether by densitometry or by psycho-physics. At any rate our measurements have failed to detect it, and are best explained without it.

Cone pigments measured by densitometry show a double-density of 0·3 or less. Measured by the break-down of metameric matches (Brindley, 1953, 1955; Walraven & Bouman, 1960; Walraven, 1966; Enoch & Stiles, 1961), a density of about 1·0 has been strongly inferred. One way to account for this very large discrepancy is to suppose (Rushton, 1958*a*) that densitometry measures pigments in the 'shallows' and psycho-physics in the 'depths' of the pigment pool. That escape is not easy to reconcile with the results of this paper. Nearly everyone agrees that rays falling obliquely upon cones are less effective because they are less well directed upon the outer segment where the visual pigment lies, less well contained there in the passage down and less well absorbed by the pigment molecules at the sites where the process of vision is initiated. We have found that obliquity of incidence of rays upon the cones affects the efficiency of vision in exactly the same way that it affects the efficiency of bleaching at all the levels we can measure. So those geometrical and optical factors that modify the quantum catch which leads to vision, modify identically the catch which bleaches the molecules we measure. Could this happen unless the quanta were caught at the same place?

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