J. Physiol. (196)5, **176**, *pp.* 462–472
With 6 text-figures
Printed in Great Britain

ROD–CONE INDEPENDENCE IN THE AFTER-FLASH EFFECT

BY M. ALPERN*
From the University of Michigan, Ann Arbor, Michigan, U.S.A.

(*Received* 26 *August* 1964)

It has been long recognized that there are three cone mechanisms that serve daylight vision and a separate rod mechanism for twilight, and now by microspectrophotometry different photosensitive pigments have been found in three types of cones in addition to the rhodopsin in rods (Marks, Dobelle & MacNichol, 1964; Brown & Wald, 1964). But it is not easy to know in various visual conditions when these four mechanisms combine and when they act independently.

In classical photometry, for instance, the contributions from the three kinds of cones are held to add to give brightness, but each is contrasted with other types to give colour. Rods and cones in conditions where both are active have been supposed to sum their contributions to brightness and to inhibit each other in some conditions of adaptation.

Stiles (1949, 1953, 1959), on the other hand, has found almost complete independence between rods and each of the three cone mechanisms in his extensive measurements of increment threshold. When a test flash of one wave-length λ was superimposed upon a background of another wave-length μ, he found that the threshold for the rod mechanism π_0 and for each of the cone mechanisms π_1, π_4, and π_5 followed the Weber–Fechner relation quite simply. For each mechanism the threshold was lowered in proportion to its sensitivity to λ and raised in proportion to its sensitivity to μ. Four independent increment threshold curves therefore co-existed, and the one that determined the threshold in any condition was that which was lowest in that condition. A similar independence among the four mechanisms was found by du Croz & Rushton (1963) in the rise of threshold during dark adaptation. For each mechanism π_0, π_1, π_4, and π_5 the rise of log threshold above the dark adapted value is proportional to the amount of pigment bleached in that type of receptor and independent of the other receptors.

Now in all the cases just mentioned the various receptors which did or did not interact lay side by side in one region of the retina. The after-flash effect (metacontrast) is a phenomenon where the interacting elements

* Assisted by NSFG 10045 from the National Science Foundation.

may be separated both in space and in time. One example of it that has been studied (Alpern, 1953) is when a test flash of 5 msec duration is followed by a second flash (also 5 msec) falling upon a separate retinal region. It was found that the presence of the second flash reduced the brightness of the test flash that preceded it and that the maximal effect occurred when the interval was some 50 msec. Now in that experiment the maximum effect occurred when the test flash excited only rods and the after-flash excited cones as well as rods. It thus seemed possible that rod latency might be so much longer than cone latency that the two receptors' activities might arise simultaneously when the two exciting lights were 50 msec apart. If this were the explanation of the phenomenon, it would be an example of inhibition of rods by cones, and one capable of further analysis.

Now there are three ways in experimental psychophysics by which rods and cones may be distinguished, (*a*) spectral sensitivity, (*b*) Stiles–Crawford effect, (*c*) rate of recovery in dark adaptation. All these have been used in the present paper to analyse the nature of interaction in this case of metacontrast. So far from rods being inhibited by the cones excited by the after-flash they are quite unaffected by them. Interaction occurs only between rods and rods or between cones and cones.

METHODS

To isolate a pure rod response to the test flash even at high light levels the two-colour method of Aguilar & Stiles (1954) has been adopted. In Fig. 1*a* the spatial arrangements of test, background and after-flash are shown. A green test flash (λ) (dominant wave-length 527 mμ) made up of a 2·5° square was exposed for a duration of 5 msec in the inferior field 6° from the fixation point. This test flash was exposed against a steady circular background (μ) (dominant wave-length 625 mμ), which extended 10° in diameter and included the parts of the field occupied by both the test flash and the fixation point (*FP*) although concentric with neither (see Fig. 1*a*). The luminance of this background could be varied from zero to a maximum of 790 scotopic trolands in 0·3 logarithmic steps by means of neutral density filters (Wratten 96 cemented in B glass), the largest density used being 6·0. The metacontrast was produced by a 5 msec after-flash, ϕ, falling upon a 9° circular field concentric with the background and homogeneous except for a blacked-out region in the part of the field where the test flash had been exposed. This flash was delivered 50 msec after the test flash and with a dominant wave-length and a luminance which could be varied respectively by placing suitable narrow band interference and neutral filters into the appropriate light beam.

The apparatus is illustrated in Fig. 1*b*. It is a three-channel Maxwellian view optical system (one channel each for the background (μ), the test (λ), and the after-flash (ϕ) fields, respectively). Each channel contains in succession a light source (*S*), a condensing lens (*L*) which imaged the sources on the aperture stop (*A*), a second set of lenses (*L'*) and a field stop (*FS*). The interference and neutral filters were mounted in appropriate places (*F*) in each beam. The system of rotating disks (*I* and *II*) and electromagnetic shutters allows the test and inducing fields to be exposed for proper durations (5 msec) and asynchrony (50 msec) in sequential series every 4 sec. The test flash luminance was varied by calibrated rotating crossed polaroids for continuous variation and with neutral filters for step changes. Fixation

was provided by a miniature-filament tungsten light (*FP*) mounted in the plane of the field stop (FS_2 or in some cases at FS_3).

After full dark adaptation the observer made three measurements of the threshold of visibility of the test flash, seen against a completely dark background. He then adapted to the lowest level of background luminance and repeated the measurements. He then adapted to the next highest level and made three more measurements and in this way a complete t. v. i. (threshold against intensity) curve was obtained. In general, the plotted points are the mean (± S.E. of the mean) of the log results of five such curves (i.e. of fifteen repetitions of the experiment).

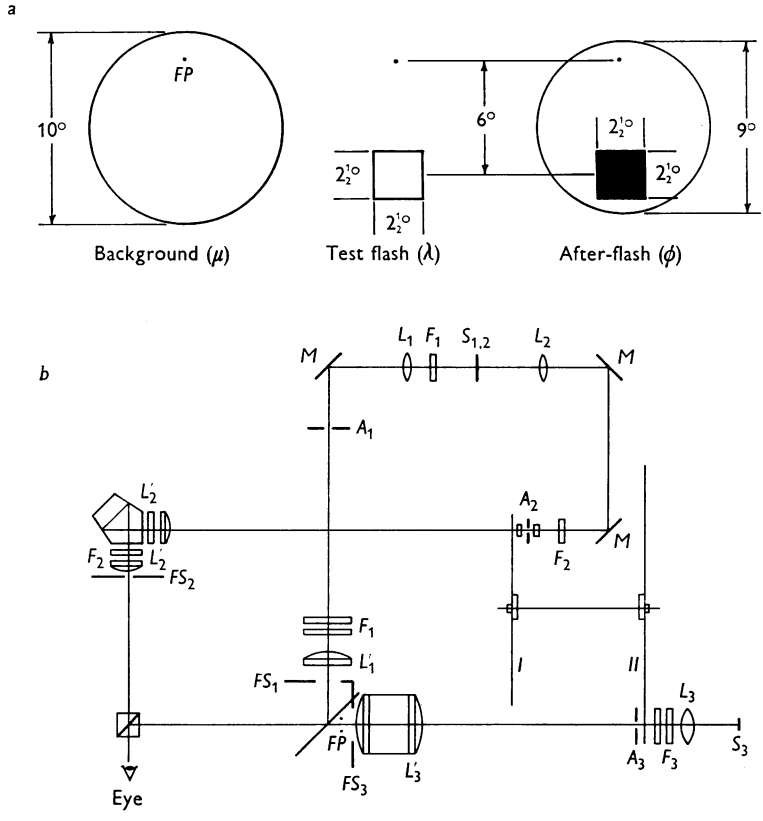

Fig. 1. The lower figure, *b*, is a schematic diagram of the apparatus. The subscripts represent the channels providing the background (1), the test flash (2), and the after-flash (3). The upper figure, *a*, shows the spatial relations of the test, background, and after-flash with respect to the fixation point (*FP*).

RESULTS

Figure 2 (white rectangles) shows the result with zero after-flash. This is simply an increment threshold curve with green flash upon a red background similar to the experiment of Aguilar and Stiles. Although the test flash area is smaller than that used by Aguilar and Stiles and

advantage was not taken of the Stiles–Crawford effect, the results in Fig. 2 show no cone branch even at the highest level studied (790 scotopic trolands). The linear part of the curve which has a slope of 1·0 stops just short of the onset of rod saturation.

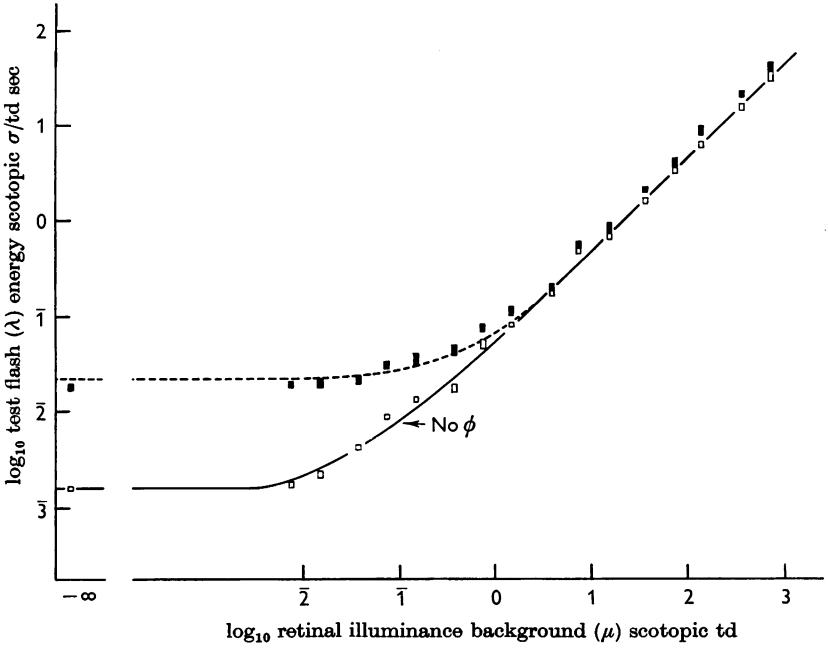

Fig. 2. The white rectangles show the results of the repetition of the Aguilar and Stiles experiment. The black rectangles show the results of the same experiment modified by presenting a fixed green after-flash that followed the test flash by an interval of 50 msec. All flash durations 5 msec. The background was red, the test flash green. 6° parafovea; λ, 527 mμ, 2·5°; μ, 625 mμ, 10°; ϕ, 500 mμ, $\bar{2}$·56 $\log_{10}$ td sec, 9°.

The after-flash effect is seen in the black rectangles of Fig. 2. The test flash and background are precisely as before, but the test was followed 50 msec later by a fixed after-flash ϕ of wave-length 500 mμ and strength $\bar{2}$·56 log td sec. The after-flash covered a 9° field homogeneous except for a black 2·5° square at the place where the test flash appeared (see top of Fig. 1).

When the background μ was very weak, this after-flash ϕ raised the threshold about 1 $\log_{10}$ unit, but with brighter backgrounds the effect of ϕ diminished and with strong backgrounds it became negligible. The nature of this effect will be studied in a later paper; the present work is devoted to the determination of the receptors involved.

Now if the intensity of the after-flash had been below the cone threshold

there could be no question but that rods alone must have been excited by ϕ as they undoubtedly were by λ. But actually ϕ lay about 1 log unit above cone threshold and the after-flash (500 mμ) was clearly seen to be coloured blue-green. Thus the question arises whether it is the excitation of the rods or of the cones by the after-flash that raises the threshold in Fig. 2 from the lower curve to the upper. The matter is easily settled by changing the after-flash ϕ to a light of different colour but the same scotopic brightness. If rods only are involved the results will be identical; if cones are involved, identity would be extremely improbable.

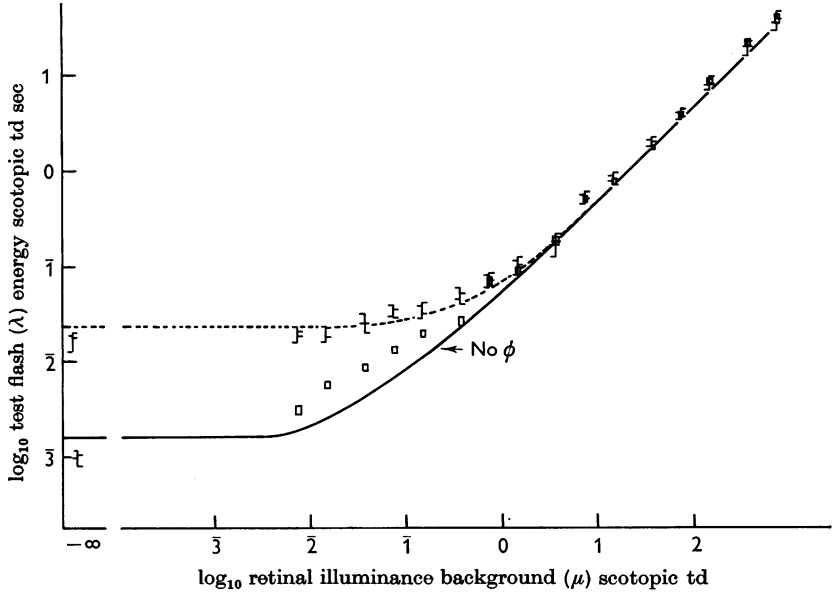

Fig. 3. The brackets open to the right ([) show the same data as the black rectangles of Fig. 2. The brackets open to the left (]) and the white rectangles show measurements when the after-flash is red and respectively scotopically and photopically equated to the green after-flash. 6° parafovea; λ, 527 mμ, 2·5°; μ, 625 mμ, 10°;]ϕ, 625 mμ, 9°; [ϕ, 500 mμ, 9°; □ϕ, 625 mμ, 9°.

In Fig. 3, the right-faced bracket ([) is a replot of the black rectangles in Fig. 2 where ϕ was of wave-length 500 mμ. The left-faced bracket (]) shows the results of changing ϕ to 625 mμ but keeping the scotopic brightness the same. The white rectangles (from a second experiment where the intensity of ϕ was reduced 30-fold) show the effect of keeping the *photopic* brightness the same. Obviously rods, not cones, must be equally stimulated by the after-flash to affect equally the threshold of the rods under the test flash λ.

The results so far apply to one fixed scotopic luminance level of the after-flash. When that is varied, the effect in raising the threshold for the test flash λ is shown in Fig. 4 for the particular case where the background is dark. (Note: in Fig. 4 it is the intensity of the after-flash, not the background, that is plotted horizontally on a log scale.)

The effect of increasing the brightness of ϕ is naturally to raise the test threshold. What is less expected is that the curve displays two or even three branches. The lowest is that already considered; rods are excited by λ and their threshold is raised as a result of *rods* excited by ϕ.

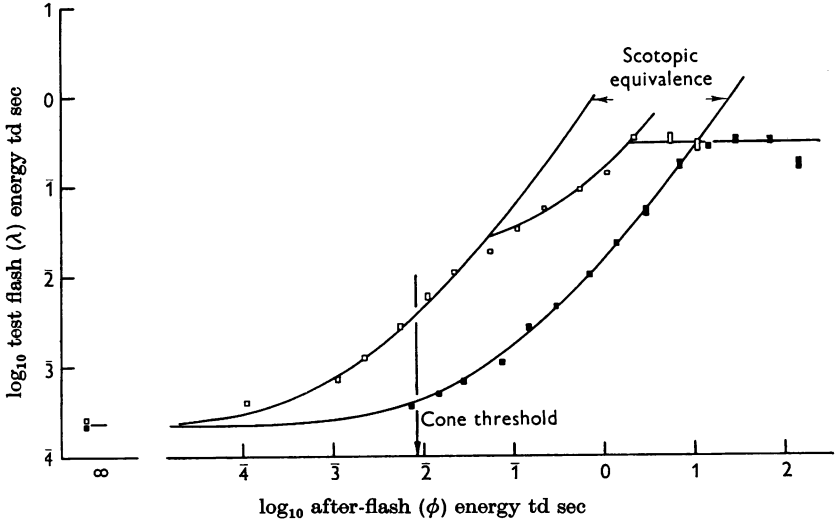

Fig. 4. Effect of luminance variation of the red (black rectangle) and green (white rectangle) after-flash upon the test flash threshold against a dark background. Note the abscissa in this figure is the after-flash energy. The smooth curves drawn through the low intensity data are empirical, but they both have the same form and are displaced horizontally by an amount equal to the difference in scotopic transmittance of the red and green filters employed. λ, 527 mμ; $\mu = 0$; □ ϕ, 500 mμ; ■ ϕ, 625 mμ.

When the after-flash was red of 625 mμ (black rectangles, Fig. 4), the rise in rod threshold could be followed for 3 log units until a 'ceiling' was suddenly reached. When ϕ was 500 mμ (white rectangles) an intermediate branch appeared between rods and ceiling. It is of interest to know the nature of the branch and the nature of the ceiling.

Now rods may easily be removed from activity by strong light adaptation as is seen in the left part of Fig. 5. The bleaching exposure was 6·92 $\log_{10}$ td sec (which bleaches about 60% rhodopsin), it was delivered through the background field and hence covered the area both of test and of after-flash. In Fig. 5 the dots on the left show the dark-adaptation

curve following this exposure measured by the same test flash λ as in Fig. 4. It is seen that between 3 and 13 min of dark adaptation the cone threshold has returned to normal but the rod threshold still lies above that of the cones. During this period therefore the experiment of Fig. 4 (ϕ = 500, white rectangles) may be repeated with the assurance that those features which are due only to cones will be preserved unchanged, but those due only to rods will be altered or abolished.

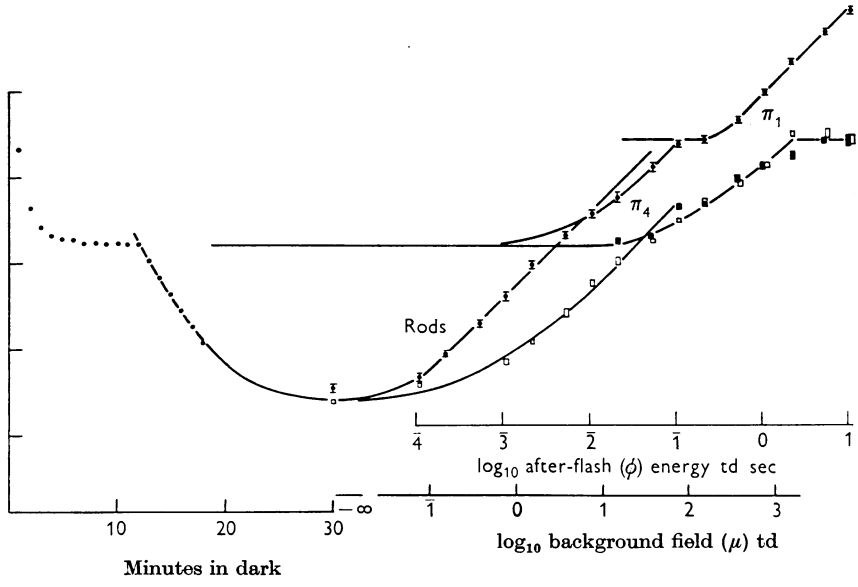

Fig. 5. The ordinates of all curves show the threshold energy of a green test flash. The left-hand curve (dots) is the mean of five dark adaptation curves following a 60 % rhodopsin bleach. The middle curve (ϕ) shows the t. v. i. curve measured against a yellow background. The white rectangles are the same as the white rectangles from Fig. 4 made after full rod adaptation. The black rectangles show the results when the experiment is repeated between 3 and 13 min in the dark after a 60 % rhodopsin bleach when the cones have fully regained sensitivity but the rods are still insensitive.

In Fig. 5 the black rectangles on the right show the result of this rod-free determination, the white rectangles simply replotting the results of Fig. 4 for comparison. It is seen, as expected, that with black rectangles the lowest branch (rods) is absent, but the two upper branches are virtually unaltered. They are therefore due to cones, and to cones only. To the question 'Which cones?' the method of Stiles provides the answer.

Stiles has shown upon the fovea that if the increment threshold curve is plotted using a green flash and a yellow background, at low background intensities, naturally, the green mechanism π_4 is excited, its threshold

being raised by increasing brightness of the yellow background. But this background that raised the threshold for both green and red mechanisms is nearly without effect upon the yellow-insensitive blue mechanism π_1. Consequently a point will come (as the background increases) when green and red thresholds rise above that for blue. Exactly this is shown in the middle curve of Fig. 5.

The curve plots the increment threshold with a flash of 527 mμ (as before) and a yellow background of 575 mμ. The region excited by λ is not the fovea, hence at the lowest background intensity rods appear. Starting then at the absolute threshold for rods (the termination of the dark adaptation curve on the left of Fig. 5) the rod increment threshold rises along the usual curve similar to that in Fig. 2. At a background of 0·5 log td the curve reaches the horizontal level of the cone dark adapted threshold. With the green test flash used this is the π_4 threshold. If the yellow background of 0·5 log td had left π_4 unaffected, the rod branch would yield to a horizontal π_4 branch at this point. But at this background the π_4 increment threshold is already beginning to rise and the rod threshold reaches it at a slightly higher level. The curve now follows π_4 for about 1 log unit and at about 2 log td of background a new branch in the curve appears which Stiles has proved to be the blue mechanisms π_1. This is so insensitive to yellow that the background has to be 100 times as strong as for π_4 to induce any threshold rise.

From the middle curve of Fig. 5 therefore we have a clear interpretation of the three branches as *rods*, π_4, π_1, and we know the absolute threshold for each mechanism. Now, turning to the after-flash curve on the right, it is clear that those three branches are the same and have the same thresholds. Thus we may conclude that in Fig. 4 (white rectangles), as the intensity of the after-flash ϕ is increased, the first effect is to raise the threshold of the rods under the test flash λ until it exceeds the threshold for the green cones π_4. But these in their turn have the threshold raised by increasing ϕ until it exceeds that for the blue cones π_1. The range studied showed no effect of ϕ upon the threshold of the blue cones. It is easy to see why the black rectangles in Fig. 4 have no π_4 branch. In this experiment ϕ was at 625 mμ instead of 500 and the log intensities plotted are photopic values. The *scotopic* value of the red light is thus some 30 times less and consequently the 'black' rod branch of Fig. 4 (which upon a scotopic plot would coincide with the 'white') should lie about 1·5 log units to the right, as is seen to be the case. This applies to the rod branch, but not to the red or green cone branches; they should remain nearly undisplaced. Thus the π_4 branch is not revealed in the curve of black rectangles because it is situated more or less where it is seen on the white curve and the 'black' rods are more sensitive throughout. In fact a red

after-flash acts very much in the same way as a red background (Fig. 2) to raise the thresholds of π_4 and π_5 relative to rods.

We have already reached the conclusion that when the test threshold λ is raised by the after-flash ϕ, rods affect rods and cones affect cones and there is no rod–cone interaction. This conclusion was established by two of the methods for discriminating between rods and cones, namely, the change in spectral sensitivity and the difference in recovery time after bleaching, and it may be further confirmed by the third method—the Stiles–Crawford effect. As Flamant & Stiles (1948) showed in man and Donner & Rushton (1959) in the frog, cones are sensitive but rods far less so to the angle of incidence of light upon them.

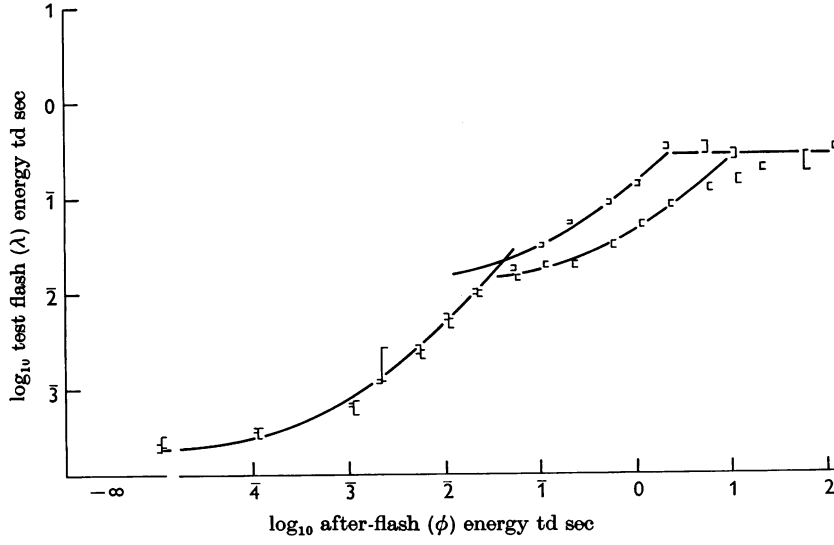

Fig. 6. The brackets open to the left (]) are the same data as the white rectangles of Fig. 4 and were taken with both test and after-flash entering the eye through the centre of the pupil. The brackets open to the right ([) are taken with the same test and after-flash but with the former entering the eye through the centre and the latter 3·5 mm temporal to the centre at the edge of the widely dilated pupil. λ, 527 mμ; μ, 0; ϕ, 500 mμ;], central pupil entry of ϕ; [, 3·5 mm temporal pupil entry of ϕ.

In Fig. 6 the results (]) of Fig. 4 are replotted and new results ([) added to show the difference when the after-flash ϕ entered the eye near the edge of the pupil (dilated with 3 drops of 1 % bis-Tropamide) instead of through the centre. The test flash still entered centrally. Since the lowest branch of the curve is unaffected, it depends only upon the rods excited by the after-flash. Since the middle branch is displaced 0·75 log unit, it depends upon the cones excited by the after-flash.

DISCUSSION

The suggestion (Alpern, 1953) that in the after-flash effect we have to do with interaction of cones excited by the after-flash and rods excited by the test flash was made on the basis of preliminary experiments which showed that: (1) maximum effects occurred at low test flash luminance levels combined with high after-flash levels, and (2) minimal effects occurred when the test and after-flash were both confined to the excitation of the rod-free fovea. Subsequent experiment has shown, however, that appreciable effects can be obtained by exclusively foveal excitation, although the time characteristics of such effects and those obtained by parafoveal excitation may well be different.

The present experiments rule out the possibility of rod–cone interaction as a basis of the after-flash effect. They show that it is the excitation of rods by the after-flash which causes the elevation of the test-flash threshold when that is determined by rods and, conversely, it is the excitation of the cones by the after-flash which causes the elevation of the test-flash threshold if the latter is determined by cones. What the mechanisms may be that produce these effects requires further study, but there is one simple question which arises directly from the present work: Does this independence, whereby rods interact only with rods and cones interact only with cones, extend to the separate classes of cones, so that π_1, π_4, and π_5 will not interact except with members of their own class? The following paper shows that this is indeed the case.

SUMMARY

1. The threshold for a 5 msec flash can be greatly raised by *following* it (50 msec later) by a 5 msec after-flash applied to the surround.

2. When the test flash excites only rods, after-flashes of various wavelengths but of fixed scotopic brightness all raise the test threshold equally. Thus it is the excitation only of the rods by the after-flash that raises the rod threshold of the test flash.

3. During the period of dark adaptation, when the cones have fully recovered but the rod threshold still lies above them, a similar effect of the after-flash in raising the test threshold may be seen. Since in this case neither flash falls upon an active rod mechanism, the phenomenon demonstrates that cones affect cones as much as rods affect rods.

4. This conclusion is confirmed by experiments in which the test flash enters through the centre, and the after-flash through either the centre or the edge, of the widely dilated pupil (Stiles–Crawford effect).

5. There was no interaction between rods and cones.

I am very much indebted to W. A. H. Rushton, for his very many helpful suggestions in carrying out these experiments and in the preparation of the manuscript.

REFERENCES

AGUILAR, M. & STILES, W. S. (1954). Saturation of the rod mechanism of the retina at high levels of stimulation. *Optica acta*, **1**, 59–65.

ALPERN, M. (1953). Metacontrast. *J. opt. Soc. Amer.* **43**, 648–657.

BROWN, P. K. & WALD, G. (1964). Visual pigments in single rods and cones of the human retina. *Science*, **144**, 45–52.

DONNER, K. O. & RUSHTON, W. A. H. (1959). Rod–cone interaction in the frog's retina analysed by the Stiles–Crawford effect and by dark adaptation. *J. Physiol.* **149**, 303–317.

DU CROZ, J. J. & RUSHTON, W. A. H. (1963). Cone dark-adaptation curves. *J. Physiol.* **168**, 52*P*.

FLAMANT, F. & STILES, W. S. (1948). The directional and spectral sensitivities of the retinal rods to adapting fields of different wave-lengths. *J. Physiol.* **107**, 187–202.

MARKS, W. B., DOBELLE, W. H. & MACNICHOL, JR., E. F. (1964). Visual pigments of single primate cones. *Science*, **143**, 1181–1183.

STILES, W. S. (1949). Increment thresholds and the mechanisms of colour vision. *Docum. ophthal.* **3**, 138–163.

STILES, W. S. (1953). Further studies of visual mechanisms by the two-colour threshold technique. *Coloquio sobre problemas opticas de la vision*, pp. 65–103. Madrid: Union internationale de physique pure et appliquée.

STILES, W. S. (1959). Color vision: the approach through increment-threshold sensitivity. *Proc. nat. Acad. Sci., Wash.*, **45**, 100–114.