

THE SITE AT WHICH RHODOPSIN BLEACHING RAISES THE SCOTOPIC THRESHOLD

H. B. BARLOW¹ and D. P. ANDREWS²

School of Optometry, University of California, Berkeley, California 94720

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THE THRESHOLD θ_t at time t during the scotopic phase of dark adaptation appears to be related to the fraction r_t of rhodopsin in the rods that is still bleached by the relation $\theta_t/\theta_0 = \exp(\alpha r_t)$, where θ_0 is the fully dark adapted threshold and α is a constant which has a value of about 44 for human rods (DOWLING and WALD, 1958; DOWLING, 1960; RUSHTON, 1961). The mechanisms by which threshold is elevated above its final fully dark-adapted value, and the site at which these mechanisms operate, are not known. Naturally the change was first thought to occur in the receptors, where the bleached pigment is, but the experiment of RUSHTON and WESTHEIMER (1962) appeared to rule this out.

They compared dark adaptation curves obtained after preadaptation to either a uniform bleaching field or a bleaching field which delivered the same total amount of light, but was spatially non-uniform. The non-uniformity was coarse, consisting of a grating with a period of 1° for the results illustrated in their paper. They found that the scotopic dark adaptation curves for uniform and grating bleaches matched when the amounts of light in the bleaching lights were the same, provided the grating period was not greater than a certain limit which was usually $30'$ but was as great as 1° for one subject. They argued that the receptors in the dark bars of the grating were weakly bleached compared with receptors exposed to the uniform bleaching field; hence, if the threshold depended only on the local concentration of rhodopsin, the test flash would have been visible on the bars of the grating at a much lower intensity than when a uniform bleach had been applied, and the failure to observe such an effect showed, they said, that the threshold of a rod can be elevated by bleaching the rhodopsin of its neighbors. This was an important conclusion and supported the view that adaptation occurred central to the receptors, where signals are pooled. RUSHTON (1965a, b) has also claimed to show that signals proportional to r , the fraction bleached, are pooled, whereas the "noisy receptor" hypothesis (BARLOW, 1964) requires that the signals should be proportional to $\exp(\alpha r)$, the dark light. For these reasons we attempted to test directly the conclusion that all the receptors are uniformly desensitized following a grating bleach, and we report this experiment here.

First some details of their original experiment must be described. The eye was fixated voluntarily in the required position, and the bleaching exposure was a brief flash to avoid blurring of the grating. The test stimuli were arranged to fall in the bleached area, but were large (3° dia.) and thus covered several bars of the bleached grating (period 1° or less). In

¹ Present Address: Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

² Present Address: Department of Communication, Keele University, Keele, Staffordshire, England.

order to make the spatial distribution of excitation resulting from the test flash similar following uniform and grating bleaches, a grating was interposed in the test beam following the uniform bleach; to compare dark adaptation curves they plotted the average luminance of the test target at threshold. What is important in their experiment is that, because of the uncertainty of fixation position (BARLOW, 1952; STEINMAN, 1965), they could not confine a stimulus to the bleached or unbleached portions of a non-uniform bleach; for this reason they had to use the large test flash covering several bars and stripes of their grating and rely upon their indirect argument. By using a stabilized retinal image we hoped to overcome the uncertainty of fixation position and thus test the threshold of bleached and unbleached bars separately. We found big differences, and this result fundamentally alters theoretical interpretations that have been based on results of non-homogeneous bleaches.

METHODS

Our targets were seen in stabilized vision, using DITCHBURN'S (1963) arrangement in the apparatus previously described by TELLER, ANDREWS and BARLOW (1966), and also used for the experiments of BARLOW and ANDREWS (1967). An unstabilized fixation point ensured that the target field remained within the aperture of the system. The contact lens was seated on the limbus and held in place by suction. It had an integral 2 mm artificial pupil, a rubber sucker and a side arm to hold the mirror. The lens was described in TELLER, ANDREWS and BARLOW (1966), but in our present experiments a short tube protruding from the center of the lens was added. This prevented smearing of the lens by blinking, which can otherwise cause a variation of image clarity from moment to moment.

The pattern of bleaching was impressed with the grating stabilized on the retina and illuminated for 30 sec at the maximum brilliance that the apparatus could deliver. The exact fraction of rhodopsin bleached is not important, but it appears from the results that it was about 40 per cent in the bleached stripes.

The threshold stimulus was a thin line, presented in the second channel of the stabilized viewing apparatus. The test line was green (Ilford 623) and was presented for 10 msec at intervals of 1.5 sec; it subtended about $1' \text{ arc} \times 1^\circ$, and was intended to be centered upon the bleached or unbleached stripes of the grating pattern.

In order to place the test line accurately upon the grating, the slit was moved by micrometer traverse; the movement was calibrated before the experiment with a viewing telescope in place of the eye so that the line could later be traversed precisely from the center of a bleached stripe to the center of an unbleached stripe or back again.

Threshold was measured at intervals of about 40 sec. The subject varied the intensity of the test flashes by means of a circular wedge, and grunted when satisfied that the stimulus was at threshold; the experimenter then recorded time and wedge setting. The position of the test line was alternated after every second trial. After each setting, the experimenter moved the circular wedge to an arbitrary position, but in the general neighborhood of the previous threshold for that stimulus location. Thus the subject had no tactile memory of the previous reading.

RESULTS

Figure 1 illustrates some results; at the beginning of the scotopic phase of each run, the threshold was found to be higher on the bleached stripes of the retina. Moving the test line by $15'$ arc caused a large drop in threshold, the maximum ratio being about 10. Obviously the rods in the dark bars of the bleach are much more sensitive.

In one run there was a reversal of the thresholds after about 20 min, and this was almost certainly caused by slippage of the contact lens. It is extremely difficult to maintain exact positioning of the contact lens for 40 min, and movements commonly occurred. They cannot, however, be held to invalidate the main conclusion we draw, because it is clear that a movement of $15'$ arc by the test line caused large changes in threshold, whereas all the points should lie on a single curve if, as Rushton and Westheimer concluded, all rods contributing to the pool are equally desensitized.

Clearly the rods in dark bars of the preadapting grating image are not only bleached less, but also have their thresholds elevated less than the neighboring rods in the bright bars.

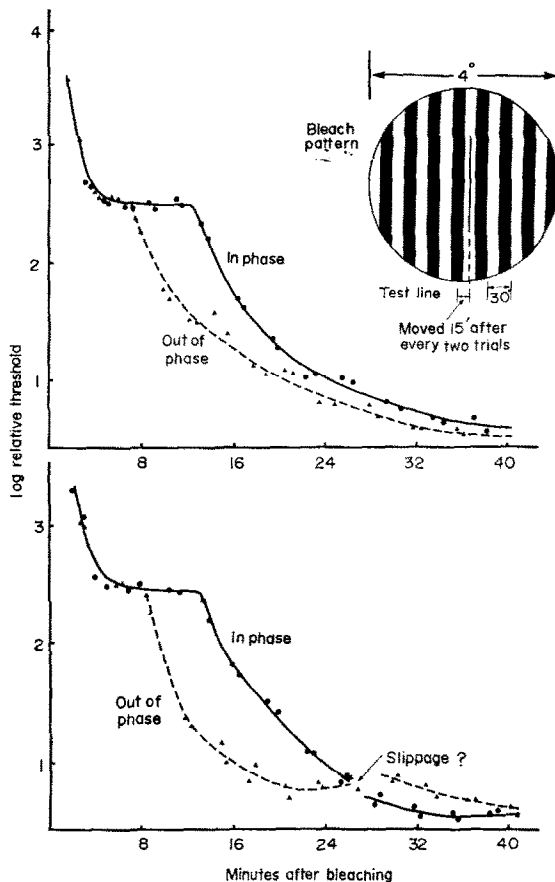


FIG. 1. Dark adaptation curves following preadapting bleach with 30 min period grating. The test target was a narrow line 1° long viewed in stabilized vision and positioned to fall on a bleached (●—●) or unbleached (▲—▲) bar of the preadapted area. The position was shifted 15 min of arc after every second threshold determination. It is difficult to maintain correct positioning for 40 min, and slippage of the contact lens is presumed to cause the result shown in the lower pair. Despite this difficulty, 15' movements of the test light certainly caused large changes of threshold; therefore threshold is not uniformly elevated within a grating bleach of period 30'.

The subject of the above experiment (H.B.B.) has also been used by Westheimer in a repeat of the original, large test field, experiment, and gave the original result when 30' period grating bleach was used. Thus the contradiction between the present result and the previous conclusion does not result from a peculiarity of the subject used.

Stabilized image experiments are so difficult technically that they are rightly regarded critically, but the present result has been confirmed and extended by ANDREWS and BUTCHER (1971) using a technique that does not require a stabilized retinal image. The original interpretation of the grating bleach experiment must be reconsidered.

DISCUSSION

In experiments reported earlier (BARLOW and ANDREWS, 1967) we found that the thresholds on dark and light bars of an illuminated grating of 30' period differed by a factor

of 2 or 3. Loss of image contrast by optical imperfections would, of course, lead one to expect some elevation of threshold in the dark bars even if adaptation is entirely local, but not to a value $1/2$ to $1/3$ as high as the value on the light bars. Thus we think there is some elevation of threshold by pooled rod signals, but the pooling area is not large enough to make the threshold elevation uniform with background illumination by a $30'$ grating. The ten fold difference between bars and stripes following a grating bleach is larger; this may be because the amplifying effect of the $\exp(ar)$ relation makes the contrast of the "dark light" grating much greater than that of the real light grating, though we cannot exclude the possibility that dark light is pooled over a smaller area than real light. In either case the results of that experiment, the present experiment, and ANDREWS and BUTCHER (1971), indicate that the threshold elevation by adjacent bleached or light-activated receptors is attenuated with distance more rapidly than Rushton and Westheimer originally suggested, so that pooling does not completely "smudge" or demodulate a $30'$ period grating. The attenuation with distance may be steep initially but flat further out, for although light or bleaching within a few minutes of arc of the test line accounts for most of the threshold raising effect, ANDREWS and BUTCHER (1971) found that bleaching 2° $30'$ from the test site still had some threshold elevating effect, though it is exceedingly small.

Rushton and Westheimer's experiment with a grating bleach and Rushton's later experiment with a spotted bleach (RUSHTON, 1965a) are consistent with the hypothesis which RUSHTON (1965b) proposed: threshold is uniformly elevated by a patterned bleach of period $30'$ or less, and the threshold-elevating signals are directly proportional to the average amount of bleaching. However, this is not the only hypothesis consistent with their results. Our experiment shows that an alternative hypothesis must be found, because threshold is certainly not uniformly elevated. Moreover, the two parts of Rushton's hypothesis are interdependent; if threshold is not uniformly raised, there is no reason to suppose that threshold-raising signals are proportional to the amount of bleaching.

Under BARLOW'S (1964) noisy receptor hypothesis of dark adaptation, threshold-elevating signals are proportional to $\exp(ar)$, the dark light, instead of (r) , the proportion of rhodopsin bleached. Rushton's experiments were designed in large part to distinguish between these two possibilities. In the light of the present experiment, this important question is once again open. Some other objections to the controlling role of dark light in dark adaptation are discussed elsewhere (BARLOW, 1972).

A hypothesis along the following lines is now tenable. Dark light signals are pooled according to the weighting function indicated by the results of BARLOW and ANDREWS (1967) and ANDREWS and BUTCHER (1971). Threshold for a test light is determined by the background of pooled dark light noise against which it must be distinguished. In the case of a large test light on a striped or spotted bleach, some of the test light falls where the dark light noise is very high, and contributes little to detection. Test light falling in unbleached regions is relatively easy to detect. The noise level there depends partly on local bleaching, but substantially upon remote bleaching also: the weight per unit area is small but the noise level and area contributing are large. The net value of threshold depends on the precise shape of the weighting function. By varying its shape, the threshold raising effect could be anywhere between the average of $[\exp(ar)]$, which is much higher than the effect predicted by average bleach, and the local value of $\exp(ar)$ in the unbleached regions, which is much lower than the effect predicted by average bleach. For any such weighting function, there will be a range of bleach patterns for which threshold will correspond to average bleaching within the limits of experimental error.

This conclusion that the dark light hypothesis has not been ruled out has a disappointing element. The equal adapting effects of non-homogeneous and homogeneous bleaches appeared to be an elegant and simple generalization that might be of basic importance in understanding adaptation. Instead, it must be attributed to the coincidental cancellation of two opposing factors.

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REFERENCES

- ANDREWS, D. P. and BUTCHER, A. K. (1971). Rod threshold and patterned rhodopsin bleaching; the pigment epithelium as an adaptation pool. *Vision Res.* **11**, 761–785.
- BARLOW, H. B. (1952). Eye movements during fixation. *J. Physiol., Lond.* **116**, 290–306.
- BARLOW, H. B. (1964a). Dark adaptation: a new hypothesis. *Vision Res.* **4**, 47–58.
- BARLOW, H. B. (1972). Dark and light adaptation: psychophysics. In: *Handbook of Sensory Physiology* (edited by D. JAMESON and L. M. HURVICH), Vol. VII/4, pp. 1–28. Springer-Verlag, Berlin.
- BARLOW, H. B. and ANDREWS, D. P. (1967). Sensitivity of receptors and receptor "pools". *J. opt. Soc. Am.* **57**, 837–838.
- DITCHBURN, R. W. (1963). Information control in the visual system. *Nature, Lond.* **198**, 630–632.
- DOWLING, J. E. (1960). Chemistry of visual adaptation in the rat. *Nature, Lond.*, **188**, 114–118.
- DOWLING, J. E. and WALD, G. (1958). Nutritional night blindness. *Ann. N.Y. Acad. Sci.* **74**, 256.
- RUSHTON, W. A. H. (1961). Rhodopsin measurement and dark-adaptation in a subject deficient in cone vision. *J. Physiol., Lond.* **156**, 193–205.
- RUSHTON, W. A. H. (1965a). Bleached rhodopsin and visual adaptation. *J. Physiol., Lond.* **181**, 645–655.
- RUSHTON, W. A. H. (1965b). The Ferrier Lecture, 1962. Visual adaptation. *Proc. R. Soc. B.* **162**, 20–46.
- RUSHTON, W. A. H. and WESTHEIMER, G. (1962). The effect upon the rod threshold of bleaching neighbouring rods. *J. Physiol., Lond.* **164**, 318–329.
- STEINMAN, R. S. (1965). Effect of target size, luminance, and color on monocular fixation. *J. opt. Soc. Am.* **55**, 1158.
- TELLER, DAVIDA Y., ANDREWS, D. P. and BARLOW, H. B. (1966). Local adaptation in stabilized vision. *Vision Res.* **6**, 701–705.

Abstract—Preadapting bleaches were delivered to the human eye through gratings of 30 min period. Using stabilized retinal imagery dark-adaptation curves were determined for a thin line placed on bleached and unbleached areas. Early in the scotopic phase of dark-adaptation a tenfold difference was found between thresholds on and off the bleached stripes. This seriously changes the meaning of the results of non-homogeneous bleaching experiments: pooled signals may control threshold, but it is not equally elevated at all positions.

Résumé—On envoie dans l'oeil humain des lumières de préadaptation à travers des réseaux de période 30 min. Avec stabilisation de l'image rétinienne, on détermine les courbes d'adaptation à l'obscurité pour une ligne fine placée dans les aires décolorées ou non. Au début de la phase scotopique de l'adaptation à l'obscurité, il existe un rapport 10 entre les seuils dans ces deux cas. Ce résultat modifie sérieusement le sens des résultats des expériences de décoloration non homogène: des signaux du "pool" rétinien peuvent agir sur le seuil, mais pas de la même façon dans toutes les position.

Zusammenfassung—Menschliche Augen wurden mit Gittern, deren Periode 30 min betrug, durch Voradaptation gebleicht. Mit einem stabilisierten Netzhautbild wurden Dunkeladaptations-Kurven für eine retinale Linie einmal auf einem gebleichten Gebiet, dann auf einem ungebleichten Gebiet gemessen. Früh in der skotopischen Phase der Dunkeladaptation ergab sich eine zehnfache Differenz zwischen den Schwellen auf und neben den gebleichten Streifen. Dies ändert die Bedeutung der Ergebnisse von inhomogenen Bleichexperimenten ernsthaft: summierte Signale können die Schwelle bestimmen, aber sie wird nicht für alle Positionen in der gleichen Weise angehoben.

Резюме—Проводилась предварительная световая адаптация глаза человека к решетке с 30-минутным периодом. Были определены кривые темновой адаптации для тонкой линии, изображение которой было стабилизировано на сетчатке, проецируемой в засвеченную или незасвеченную область. В ранней скотопической фазе темновой адаптации было обнаружено десятикратное различие между порогам на включение и выключение светящихся полос. Эти данные серьезно изменяют представление о результатах негомогенных экспериментов с обесцвечиванием (bleaching experiment): суммирующиеся сигналы могут контролировать порог, но он неодинаково повышается во всех положениях.