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DARK ADAPTATION WITHIN THE RECEPTIVE FIELD CENTRE OF RAT RETINAL GANGLION CELLS

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SUMMARY

1. Recordings from single axons of retinal ganglion cells in the rat's optic tract were used to determine whether bleaching a small area of the receptive field reduced sensitivity globally or locally, near the bleached photoreceptors.

2. When a suprathreshold test spot was alternated between two equally sensitive positions, the ganglion cell gave an approximately balanced response. The balance was upset if a small-spot bleach was selectively applied to one position. Recovery of the balanced condition was rapid.

3. Varying the duration of a constant illuminance bleach varied the duration of the imbalance following the bleach.

4. The recovery of sensitivity after small-spot bleaches was measured both at the location of the bleach and also at another location, initially equally sensitive. The recovery at the bleached location lagged recovery at the unbleached location; but even in the bleached location, the return of sensitivity was rapid.

5. Recovery of sensitivity after half-field bleaches was measured in the bleached and unbleached halves of the receptive field. Recovery in the bleached half lagged that in the unbleached half.

6. A comparison between the effects of a small-spot bleach and a half-field bleach of the same strength show that the duration of dark adaptation depends on the area of the bleach.

INTRODUCTION

We have shown previously that the desensitizing effect of illuminating a small area of the receptive field of a ganglion cell is not confined to the illuminated area but spreads laterally (Green, Tong & Cicerone, 1977), although not uniformly (Cicerone & Green, 1980) throughout the field centre. Locations near the illuminated area suffer a greater sensitivity loss than locations further from illumination. These experiments provided electrophysiological evidence that the adaptation pool (Rushton, 1965*a*) can be smaller than the ganglion cell receptive field centre. Given the psychophysical evidence for the equivalence of adapting backgrounds and bleaching signals in dark adaptation (Crawford, 1947; Blakemore & Rushton, 1965*a*, *b*; Barlow & Sparrock, 1964), one might strongly suspect that these localized adapting effects could equally well be demonstrated during dark adaptation following localized bleaches.

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Experiments were designed to determine whether bleaching a small area of the receptive field reduces sensitivity globally or locally, near the photoreceptors with bleached pigment. Our results from recordings of activity in single axons of rat retinal ganglion cells show that the effects of bleaching adaptation can differentially affect localized areas within the receptive-field centre.

METHODS

The methods are as described before (Cicerone & Green, 1980) with the following exceptions and additions.

When an additional adapting source was needed, light (from a Kodak Carousel, model 800, with a 500 W lamp) was back-projected onto the tangent screen. Light from this source was modified by neutral-density filters and was varied in its spatial extent by stops that provided full-field, half-field or small-spot illumination of receptive fields. The bleaching source for most of the experiments was this projector. In a few experiments a 150 watt xenon arc lamp was the bleaching source.

The luminance of the bleaching light was measured with an SEI photometer. The photometer had been calibrated against a standard lamp (Macbeth illuminometer). A luminance measurement was made for each bleaching light as it was presented during the course of an experiment. Whenever possible, multiple measurements were made and averaged.

The following procedure was used to estimate the quantal absorptions and the pigment bleached. The retinal illumination in terms of scotopic trolands was calculated from the photometric measurements by taking into account the area of the pupil (0·79 mm^2 with the 1 mm diameter artificial pupil or 19·64 mm^2 with the fully dilated 5 mm diameter natural pupil) and the photopic to scotopic conversion for our 6000 °K xenon source (2·4) or our 3000 °K tungsten source (1·5). In man, 1 scotopic troland of 500nm retinal illumination produces 5×10^6 quanta/mm^2.sec incident on the retina. Retinal illumination is inversely proportional to the square of the posterior nodal distance. The posterior nodal distance of the rat eye is 2·97 mm (Block, 1969) as compared to 16.7 mm in man (Le Grand, 1957). The ratio of the squares of the nodal distances was applied to obtain $1{\cdot}6 \times 10^8$ quanta/mm^2.sec incident on the rat retina due to each scotopic troland. Using the figures of 4×10^5 rods/mm^2 for the rat and 25 % of the incident quanta absorbed (Cone, 1963), 100 quanta per sec is obtained as an estimate of the *absorption* by a rod for each scotopic troland of retinal illumination. The fraction of pigment bleached in a rod was calculated as $1 - e^{-It/N}$, where I is the quantal absorption per second, t is the bleach duration in seconds and N ($= 3{\cdot}2 \times 10^7$) is the number of rhodopsin molecules (Cone, 1963). For example, the bleaching stimulus used in Fig. 1 produced a photopic luminance of 685 cd/m^2 (200 ft-L) on the tangent screen. For the xenon source and the 1 mm diameter pupil, this would produce 3·11 log scotopic trolands of retinal illumination and so each rod would absorb 5·11 log quanta per sec. The fraction of pigment bleached is calculated directly as $1 - e^{-It/N} = 0{\cdot}21$, where in this case $t = 60$ sec and $I = 1{\cdot}29 \times 10^5$ quanta per sec. The above is equivalent to taking Q_e, the bleaching energy that leaves 1/e of the dark-adapted rhodopsin unbleached, to be 15·71 log *incident* quanta/cm^2. This agrees with Perlman's (1978) empirically measured value of $15{\cdot}9 \pm 0{\cdot}4$, for the normal rat.

A ganglion cell's receptive field profile was determined by placing a small 1° spot of light in various locations and measuring the light necessary to evoke a response of 6 spikes/sec above base-line firing rate (Cicerone & Green, 1980). Then a suprathreshold (10 × to 100 ×) test spot was alternated between two equally sensitive positions in the field. That is, light from one channel of the stimulator was imaged onto one position and light from another onto the second position, and these were alternately switched on and off at 1·5 sec intervals. In this way, luminances could be independently adjusted in each position until the cell's response was balanced. Balance was attained when the cell's responses to the stimuli in each position were identical, as for example, with the OFF unit at the top of Fig. 1. Then a portion of the ganglion cell's receptive field centre was bleached by illumination for 60 sec with a 'bleaching' field. The bleaching field was either a spot (1·5° diameter) coincident with one of the two test positions or a semicircle (12° radius) bisecting the receptive field. The imbalance of previously balanced responses was used as a measure of the local bleaching effect.

In addition to the above response measures, in some experiments sensitivity was measured during dark adaptation following a small spot or a half-field bleach. Two positions, previously determined to be equally sensitive, one in the bleached area and the other in the unbleached area, were tested for differential bleaching effects.

Sixteen ON units and thirteen OFF units were in studied eighteen animals.

RESULTS

Position-exchange after small spot bleaches

Fig. 1*A* shows the response of an OFF unit when a suprathreshold (10 × threshold) test spot of light alternately illuminates two equally sensitive positions in the receptive-field centre. The data shown in Fig. 1*B* and *C* are records during dark adap-

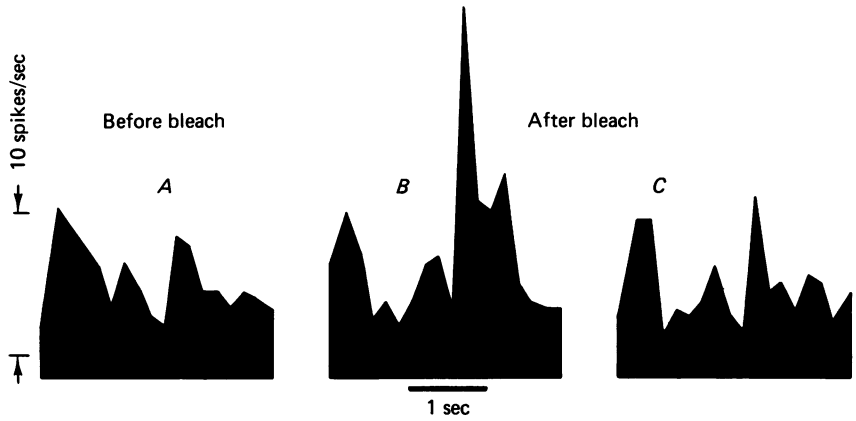

Fig. 1. The post-stimulus time histogram marked *A* shows the response of an OFF unit to alternating a 10 × threhold test spot of light between two equally sensitive positions in the receptive field. The histogram spans 3 sec. For the first 1·5 sec the test was imaged at position 1 and for the second 1·5 sec interval it was at position 2. A small-spot bleach was selectively applied at one position for 60 sec. The estimated amount of pigment bleached within the small spot was 21 %. Histogram *B* shows the marked imbalance recorded 15 sec into dark adaptation. The response in the unbleached location dwarfs the response in the bleached location. However, histogram *C* shows that the dramatic imbalance produced earlier is abolished at 135 sec after termination of the bleach. The balanced response has been re-established.

tation after a small intense spot was used to bleach one of the test positions. The bleaching stimulus was a 1·5°, 685 cd/m^2 spot which was presented for 60 secs through a 1 mm artificial pupil at the rat's eye. The stimulus was estimated to bleach 21 % of the rhodopsin in the centre of the image of the spot (see Methods). Fig. 1*B* shows that immediately after the termination of the bleaching stimulus, the previously balanced response was unbalanced. The response in the unbleached position was enhanced relative to the response in the bleached position. The enhancement was only relative since, as will be shown below, bleaching one position depressed sensitivity to some degree at both positions (see Fig. 5, for example). Fig. 1*C* shows that the dramatic imbalance which was present at 15 sec has disappeared at 135 sec. We found this rapid recovery of balance after a small spot bleach to be a consistent result with up to an estimated 60 % of the visual pigment bleached.

Rapid recovery is partly a consequence of bleach strength. If the artificial pupil is removed, thus increasing the bleach effectiveness by a factor of 25, then the recovery of balance is slowed. Fig. 2 shows post-stimulus time histograms for an ON unit before and after a small-spot bleach. The first record shows the unit's response in the balanced condition. A 60 sec, 170 cd/m^2 exposure applied to one of the test positions produced the time sequence of changes shown in the remainder of Fig. 2. The small-spot bleach (estimated to bleach 61 % of the pigment) produced a sharp imbalance.

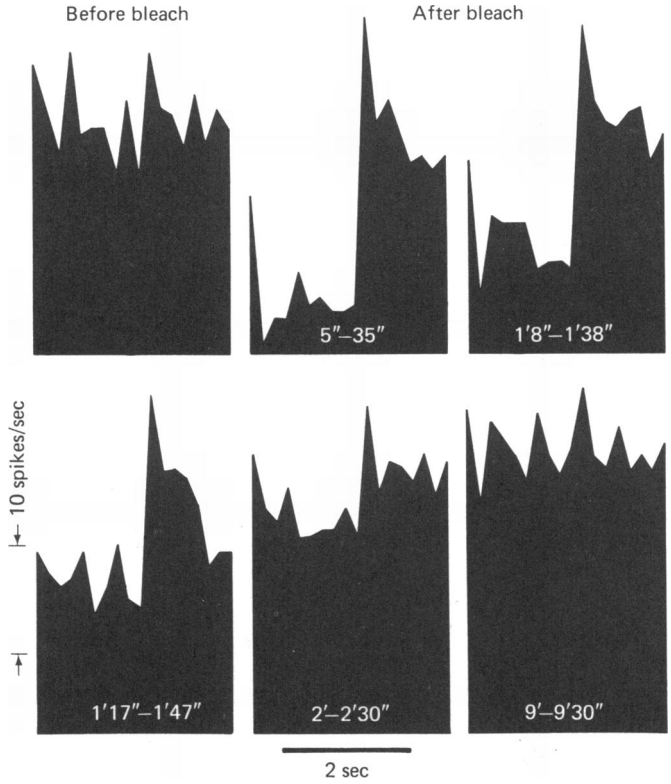

Fig. 2. The histograms show the slower progression of response balance recovery when a more intense small-spot bleach, estimated to bleach 61 % of the pigment, is applied. The first record is the response of an ON unit to a 100 × threshold test spot alternated between two equally sensitive positions in the receptive field. The next five histograms show the sequence of response-balance recovery till at 9 min the balanced configuration is regained. The numbers below the histograms record the time intervals during dark adaptation at which the histograms were taken.

Slowly the imbalance diminished until at about 9 min into dark adaption balance was restored. Table 1 shows the time needed for recovery of balance as a function of bleaching luminance for all units tested with a small spot bleach.

The balance experiments demonstrate that selective bleaching of restricted receptive field areas can produce lasting differential changes in the responses obtained by stimulating these areas. Interpretation of these results as evidence for the restricted spread of the desensitizing effects of bleaching signals is hampered by the following observations from vertebrate photoreceptors. Photovoltages in rods saturate at low

TABLE 1. Response recovery after small-spot bleaches

Unit	Type	Bleach duration (sec)	Bleach (per rod) (log quanta/sec)	Estimated pigment bleached %	Time for recovery of response balance (min)
04/13/6/1	OFF	60	5·10	21	No imbalance
04/19/6/2	OFF	60	5·10	21	2·2
04/19/6/5	OFF	60	5·10	21	3·0
06/15/6/1	OFF	60	5·10	21	0·9
06/28/6/2	ON	60	5·30	31	3·5
01/24/7/1	ON	60	4·35	4	2·5
05/17/7/2	ON	10	5·70	14	2·3
		30	5·70	37	3·8
		60	5·70	61	7·3
05/17/7/6	ON	10	5·70	14	1·6
		30	5·70	37	4·2
		60	5·70	61	8·9

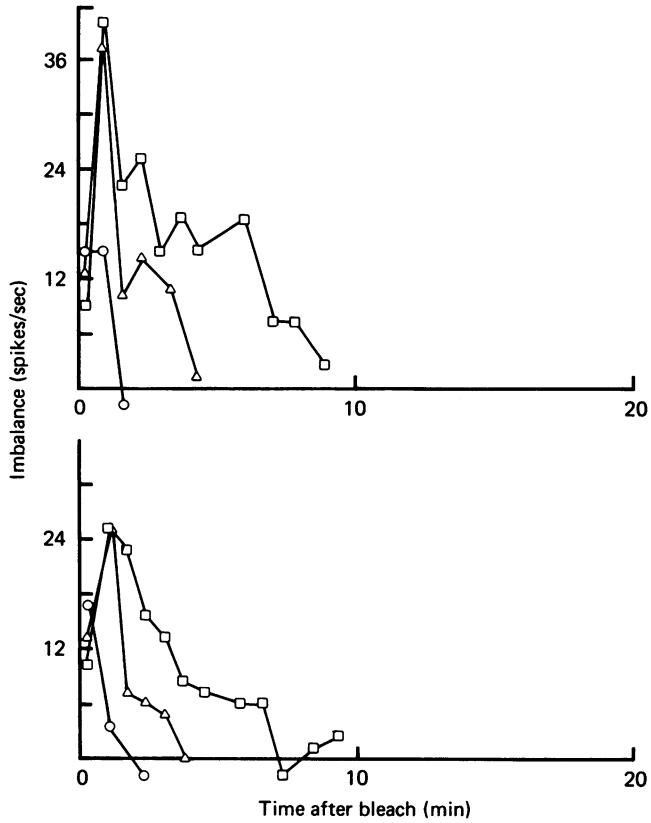

Fig. 3. Shown for two ON units are the time courses of dark adaptation after a 10 sec (○), 30 sec (△) or 60 sec (□) application of a small-spot bleaching light. The light produced an irradiance on the retina estimated to be 5·5 log quanta absorbed/rod.sec. The magnitude of the imbalance, in peak to trough differences between the unbleached and bleached locations, is plotted at various times after the bleach. The magnitude and duration of the imbalance is graded according to the bleach duration.

levels of stimulation (approximately 200 quanta absorbed per rod). As stimulation is increased the photovoltage amplitude shows little further increase but its duration is progressively prolonged. It might be argued that in our experiments the photoreceptors in the bleached area remain strongly saturated for a long time after the termination of the bleach so that even strong stimulation soon after the bleach is ineffective. Thus the preceding findings might not be due to bleaching adaptation in the usual sense, but rather to neural response-compression because of the non-linear amplitude–intensity relationship of the rods and the prolonged duration of responses to bright bleaches.

If the imbalance we find is due to rod saturation then the magnitude of imbalance should depend on the intensity of the bleaching stimulus but not on its duration. On the other hand, if rod saturation is not significant then prolonging the exposure will increase the proportion of visual pigment bleached, and the magnitude and duration of the response imbalance should be prolonged. Accordingly, the same small spot adapting luminance was applied for either 10, 30 or 60 sec. It was determined that the bleaching light caused each rod to absorb 5·50 log quanta per sec. Fig. 3 shows the results from two typical units. The magnitude of the imbalance, in terms of the peak-to-trough differences between the response to stimulation in the bleached and unbleached positions, is plotted for various times after the bleach. Fig. 3 shows that the time courses of recovery after the 10, 30 and 60 sec bleaches are not identical. The magnitude and duration of the imbalance is graded according to the duration of the bleach.

Recovery of threshold in bleached versus unbleached areas

Figs. 1 and 2 show the recovery of response after a small area of the receptive field is exposed to a bleaching light. The measurements suggest that a larger desensitization has been produced in the bleached area.

A direct comparison of sensitivity measurements and response measurements was made on the unit depicted in Fig. 4. At the top are post-stimulus time histograms showing the response of the unit to stimulation by a ten-times threshold light alternated between two equally sensitive positions in the receptive field. The left-most record shows the unit's initially balanced response to this stimulation. The subsequent histograms were recorded at 20, 60, 95 and 135 sec after a small-spot 4 % bleach was applied at one position. The balanced state is apparently restored after only 135 sec of dark adaptation. However, the sensitivity in bleached and unbleached areas is not the same, as is shown at the bottom of Fig. 4. For this same unit, if thresholds in bleached vs. unbleached areas are compared, a 10 min long difference in adaptation state is revealed.

The effects measured with small-spot bleaches are fairly small and short-lived. To obtain a more robust difference, half-field bleaches were used and ganglion cell recovery of sensitivity was tracked in bleached and unbleached half-fields. Fig. 5 shows dark adaptation curves measured after a small-spot bleach and after bleaching an area which covered half the receptive-field centre. The test lights were positioned so as to fall upon two equally sensitive locations in the right and left half-fields. At

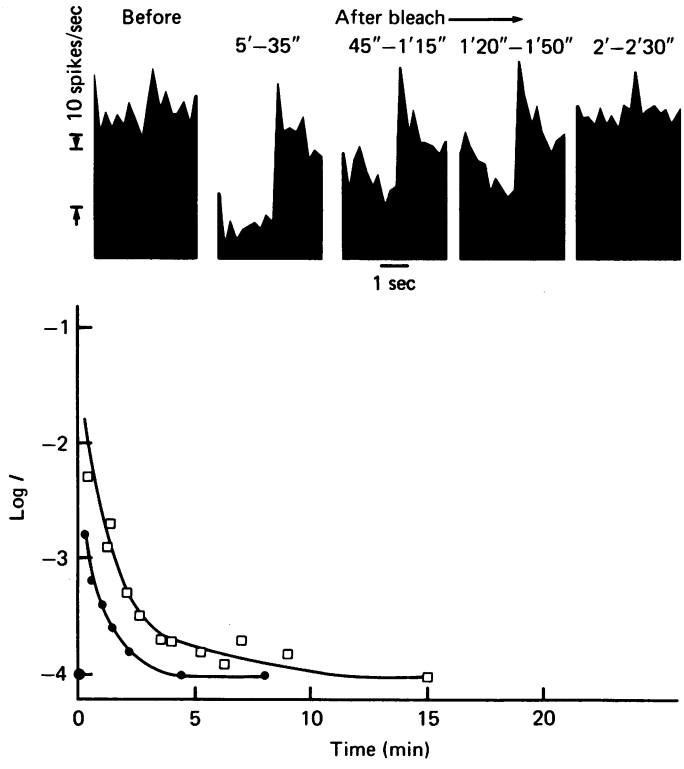

Fig. 4. At the top are shown post-stimulus time histograms of the response of an ON unit to a small spot of light alternated between two equally sensitive locations in the receptive field. The first record shows the balanced response of the unit to the light positioned at one location for 1500 msec and then at the other for the next 1500 msec. The next four records show the initial imbalance and subsequent recovery of balance after a small spot, estimated to bleach 4 % of the pigment, was selectively applied at one location. The numbers above the histograms record the time intervals during dark adaptation at which the histograms were taken. The balance is apparently restored after 2·5 min into dark adaptation. If the return of thresholds is measured in the same locations after the same small-spot bleach, the results are as shown at the bottom. The open symbols are measures taken at the bleached location, the filled symbols at the unbleached. Threshold recorded prior to the bleach is given by the symbols drawn at zero time. In contrast to the results of the response measurements, 2 min into dark adaptation, thresholds at bleached and unbleached locations are separated by half a log unit. Zero on the ordinate corresponds to a test luminance of 1·6 log cd/m^2.

the positions tested the sensitivity was 0·3 log units below maximum. After the small-spot bleach (estimated to bleach 23 % of the pigment within the area it covered) recovery of sensitivity is rapid but proceeds more slowly at the bleached location. After the half-field bleaching stimulus of the same luminance was applied, thresholds were alternately measured in each half-field. Nearly 80 min were required for recovery of the threshold in the bleached half-field. In contrast, recovery took nearly 40 min in the unbleached half-field.

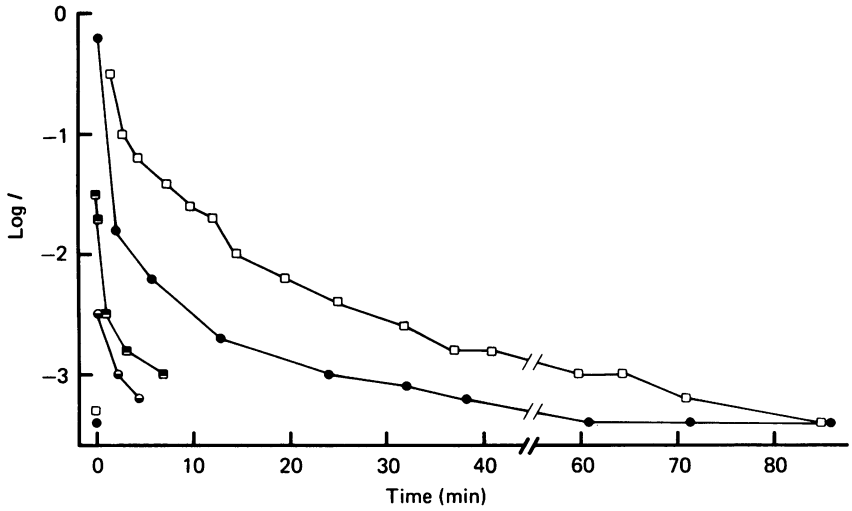

Fig. 5. Dark-adaptation curves for an OFF unit after a small-spot bleach and a half-field bleach of intensity estimated to bleach 23 % of the pigment within the areas they cover. Thresholds measured (at location ± 1·8°) prior to application of the bleaching lights are marked at zero time. The recovery after a small-spot bleach is rapid at the unbleached location (◑) and is lagged by recovery of sensitivity at the bleached location (◧). After a half-field bleach, recovery in the bleached half-field takes 80 min (□), and recovery in the unbleached half-field takes 40 min (●). Zero on the ordinate corresponds to a test luminance of 1·6 log cd/m².

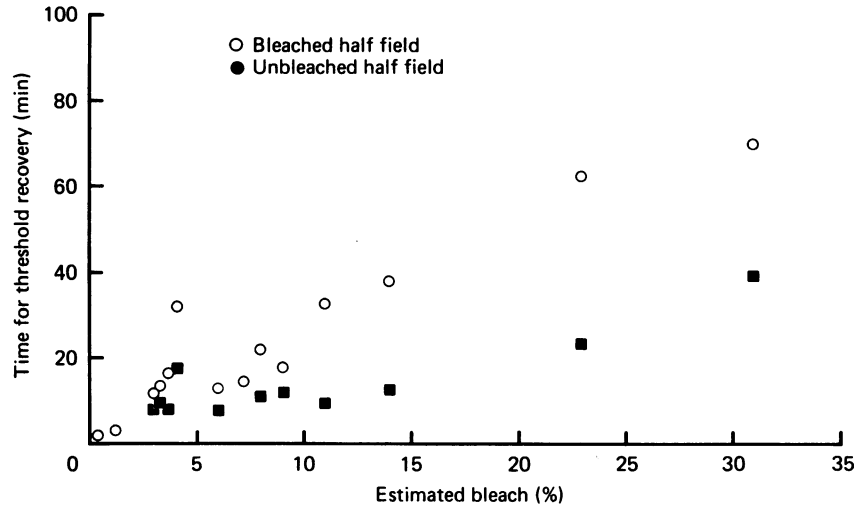

Fig. 6. Times for recovery of sensitivity after half-field bleaches of varying effectiveness. Each point (or pair of points) summarizes the results from a different unit, except that the pairs of points plotted for estimated bleaches of 7·2 % and 23 % are the mean results from two different units. ○, represent the recovery times in the bleached half-field, ■, the recovery times in the unbleached half-fields.

That the time course of dark adaptation was faster, and thresholds were consistently lower, in the unbleached half-field is shown in Table 2 which summarizes our results during dark adaptation for eleven units. In Fig. 6 are plotted the mean recovery times in bleached and unbleached locations after half-field bleaches of varying effectiveness.

TABLE 2. Threshold recovery after 60 sec. half-field bleaches

Unit	Type	Bleach (per rod) (log quanta/sec)	Estimated pigment bleached (%)	Time for threshold recovery (min)	
				Bleached half-field	Unbleached half-field
12/12/7/4	OFF	4·20	3·0	12·0	8·0
04/11/7/2	ON	4·25	3·3	13·5	9·5
01/19/7/1	ON	4·30	3·7	23·0	10·0
01/24/7/1	ON	4·35	4·1	32·0	17·5
06/22/6/4	ON	4·60	7·2	14·5	No measurements
08/30/7/1	OFF	4·60	7·2	6·0	6·0
12/12/7/5	OFF	4·70	8·0	22·0	11·0
09/06/7/2	ON	3·30	0·4	2·0	Less than 2 min
		3·80	1·2	4·0	Less than 2 min
		4·30	3·7	17·0	6·0
		4·80	11·0	45·0	13·5
07/07/6/1	OFF	5·14	23·0	47·0	8·2
07/07/6/3	OFF	5·14	23·0	78·0	38·5
08/30/7/3	OFF	3·80	1·2	2·0	Less than 2 min
		4·30	3·7	9·5	Less than 2 min
		4·80	11·0	20·0	5·5
		5·30	31·0	70·0	39·0

Relationship between the spread of adapting signals and spread of bleaching signals

The difference in the dark-adaptation curves in bleached *vs.* unbleached half-fields has been used as a measure of the spread of bleaching signals within the receptive-field centre. The substantial differences mean that complete spread of bleaching signals is more restricted than the spread of exitatory signals within a ganglion cell's receptive-field centre. In a similar way, light adaptation confined to a subarea of the receptive field has previously been shown to be most effective in areas near the adaptation site (Green *et al.* 1978). Can the extent of spread of bleaching signals be linked to the restricted spread of light adaptation?

Little differential effect in bleached and unbleached areas was found for only two units out of twenty-nine that were studied. The dark adaptation curves measured in unbleached and bleached half-fields for one such unit are shown in Fig. 7*A*. Recovery of the threshold proceeds similarly in each half-field. In contrast, the bulk of the units showed a substantial difference in dark adaptation in bleached and unbleached half-fields. An example of this latter behaviour is shown in Fig. 7*B*. Early in dark adaptation there is more than a log unit separation between the curves.

Fig. 7*C* shows for the unit of Fig. 7*A*, the results from the selective-adaptation experiments. The left-most histogram shows the balanced response to the alternation of a 100-times threshold light between two equally sensitive positions. The next record shows that an adapting spot imaged upon one of the locations caused a

decrease in the response to the test placed at the adapted position and a small relative increase to the test placed at the unadapted position. The last histogram results from switching the location of the adapting light. Again there was an unimpressive change in the response characteristic, now slightly favouring the test in

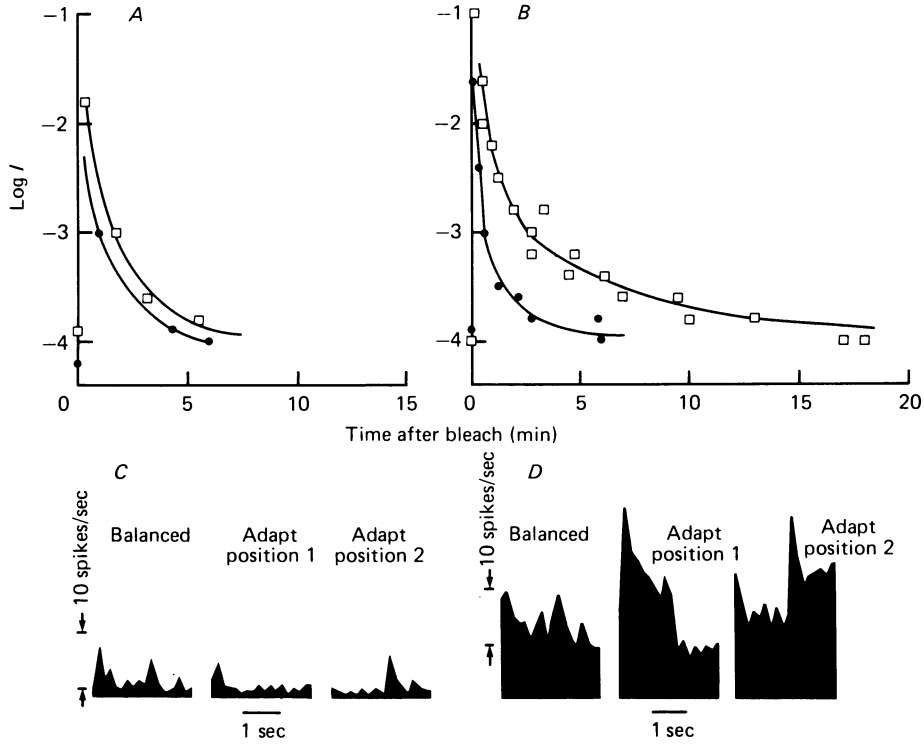

Fig. 7. *A* and *B*, the return of sensitivity after a half-field bleach for two different units. The (●) mark the thresholds in the unbleached half-field, the (△) mark the thresholds in the bleached half-field. The determinations marked at zero time are the pre-bleach thresholds. For the unit shown in *A*, where thresholds were measured at location ± 1·7°, there is hardly any differential bleaching effect. For the unit shown in *B*, there is a substantial difference of more than 1 log unit between the thresholds in the bleached and unbleached half-fields (measured at ± 1·5° in the receptive field) which diminishes in magnitude but is maintained for 15 min into dark adaptation for these same two units. *C* shows the results for the same unit as *A* and *D* for the unit *B*. The leftmost histogram results from an alternation of a 100 × threshold light between two equally sensitive locations in the receptive field. The next two records show the unit's response when an adapting light was selectively placed at one location, then the other location. A large differential adapting effect is exhibited in *D*, but not in *C*. Zero on the ordinate (in *A* and *B*) corresponds to a test luminance of 1·6 log cd/m².

position 1. Fig. 7*D* shows for the unit of Fig. 7*B* the results of the differential adaptation experiments. An adapting light placed at position 1 causes a marked imbalance and a large response to the test in position 2. Similarly, switching the adapting light to location 2 results in a brisk response to the test in position 1. In addition, threshold changes in light-adapted and unadapted half-fields were measured. Two equally sensitive locations in the receptive field were predetermined by measuring the dark-

adapted thresholds. The luminance of a half-field adapting light was adjusted to raise the dark-adapted threshold within the illuminated half-field by 100-fold. Threshold was determined at the equally sensitive position in the unadapted half-field. The thresholds differed by 0·2 log unit for the unit depicted in Fig. 7*A* and *C*, but differed by 1·2 log units for the unit depicted in Fig. 7*B* and *D*. The restricted spread of bleaching signals matches the spread of adapting signals.

DISCUSSION

The spread of bleaching effects in rat retinal ganglion cells was measured by testing at two equally sensitive locations in the receptive-field centre after applying bleaches selectively at one location. A restriction of bleaching signals to areas near the bleached photoreceptors was demonstrated by using both response and threshold measures during dark adaptation. The effect of the bleach at near positions in the receptive field was shown to be of greater magnitude and longer duration than the effect on far positions. Although our evidence points to a restriction of bleaching signals to sites near the bleached locus, it is clear that there is some spread of bleaching effects into areas not directly bleached (Figs. 4 and 5).

The nature of lateral influences in adaptation has been studied psychophysically by measuring the extent to which bleaching in distant areas can affect threshold. An early set of experiments (Rushton & Westheimer, 1962; Rushton, 1965*b*) involving threshold measurements upon bleaching fields which were either uniform or patterned (gratings or spotted) seemed to indicate that bleaching effects spread uniformly in 'adaptation pools' (Rushton, 1965*a*) of at least 30 minutes of arc. Within such pools, differences in adapting luminance seemed to cause no local variations in sensitivity. Another set of experiments (Andrews & Butcher, 1971; Barlow & Andrews, 1973) led to a modification of Rushton's idea and the conclusion that bleaching signals *are* pooled, but not uniformly. They are pooled according to a weighting function that descends sharply with distance from the bleached area. These results may be related to our findings from rat retinal ganglion cells in the following way. The weighting function of Andrews & Butcher (1971) shows a 100-fold fall off at 15 minutes of arc from the bleaching locus. This corresponds to approximately 73 μm of retinal distance in man. This retinal distance corresponds to 1·4° of visual angle for the rat. Fig. 5 shows the differences in dark adaptation measured in bleached and unbleached half-fields. The test spot in the unbleached half-field was located at 1·8° from the edge of the bleaching field. In this case, there is more than a log unit difference in sensitivity at early times between measurements made within the bleached area and at 1·8° removed.

The response of a unit to suprathreshold stimuli alternating between equally sensitive receptive field areas has proved to be an effective measure of local adaptive effects. When the balance was upset with the selective application of a bleaching light to a portion of the receptive field, restricted spread of bleaching signals was inferred, for if spread had been uniform, the balance should not have been upset. The imbalance was dramatic and convincingly demonstrated the magnitude of localized effects.

Part of our purpose in contrasting response and threshold measures of dark adap-

tation was to clarify conclusions on the spread of bleaching effects drawn from previous experiments using response measures (Bonds, 1974). We found the response measures obtained by alternating a 100-times threshold stimulus between two positions did not always accurately reflect the time course of dark adaptation in bleached and unbleached locations as did the threshold measurements. First, as Fig. 4 shows, the return of balance in our response measure occurs when sensitivities in bleached and unbleached areas are elevated 0·5 log unit above dark-adapted values. Secondly, our on-line computer allowed observations to be made immediately after application of the bleach. However, if the first observation had instead been made at 2 min, then, based on the response measure, little or no differential spread of bleaching signals might have been observed. A third problem arising from the use of responses to stimuli of fixed luminance occurs with strong bleaches as shown in Fig. 3. Early in dark adaptation the response imbalance in bleached and unbleached positions is small compared to subsequent differences. This apparently occurs because of the short-term but profound reduction of sensitivity caused by the strong bleaching light. Responses at the bleached and unbleached locations are momentarily driven to a minimum. Differential bleaching effects are therefore hidden for a time until the response regains strength at both locations in subsequent dark adaptation. These points illustrate the caution which must be applied when interpreting the results obtained from response measures alone.

The rapid restoration of response balance is a surprising finding if one accepts the estimates of bleaching. The complete regeneration of rhodopsin in the bleached area is unlikely since after strong bleaches rhodopsin in the rat regenerates with a half-recovery time of 30 min or longer (Tansley, 1931; Lewis, 1957; Dowling, 1960; Perlman, 1978). Recovery of response balance could occur because the desensitization caused by the bleach has, with time, spread so that it is equal at the two test positions. Alternatively, sensitivity could completely and rapidly recover to dark adapted levels within minutes after the small spot bleach. To distinguish between these two possibilities we tracked the recovery of sensitivity after the small spot bleach. In every unit tested sensitivity completely recovers within several minutes after bleach. (See Fig. 4). Our estimates of bleach are based on the assumption that all of the bleaching light falls within the geometric image of the stimulus. This is almost certainly not true for small spots. Bleaching is an approximately linear process so that a small error in the estimate of log quantum flux can lead to a significant error in estimated bleach. If the actual bleach with a small spot was lower than estimated then this may help to explain why recovery is so rapid.

The experiments of Fig. 3 argue against rod saturation as the major factor in our results. The argument rests on the assumption that if rods are saturated the recovery from a long flash is dependent only on the intensity of and not the duration of the prior exposure. To our knowledge, these kinds of experiments have not been done on mammalian rods. Penn & Hagins (1972) found that for brief flashes with energies of up to about 10^4 quanta absorbed per rod, the photocurrent response can be modelled by a chain of linear low-pass filters followed by a non-linear amplitude-limiting process with a hyperbolic saturation characteristic. For this system, the response to a prolonged bright light should show a rise to the maximal saturated value, a maintenance at this value after the light is turned off and then a return to base line. The linearity of the system and rate saturation predict that for a bright light whose

duration is longer than the step response, the rate of return to base line should be independent of the duration of stimulation. If the slow return of the rod potential is a major factor in our results, these predictions should be borne out. They are not. Bleaching with a bright, amplitude-saturating and rate-saturating light applied for varying durations shows a rate of return of responsivity which is graded with duration. In a study which is not entirely pertinent to our results, Steinberg (1969) showed that when 0·1 per cent of the rhodopsin was bleached with a brief flash, the S-potential in the cat returned to resting level in about 7 sec. After strong bleaches ($< 50\%$), the S-potential could take minutes to return to base line. Moreover, the time to recover half voltage of the S-potential was graded with the duration of the bleach exposure. In addition, the S-potential returned to base line well before the recovery of either cone or rod excitability. Thus, for the cat, as Naka & Rushton (1968) had found for fish, the level of potential following bleach does not determine the level of excitability. Here we deal with excitability.

If it is assumed that all quanta delivered in 60 sec are equally effective, then the longest bleach results in 7·48 log quanta absorbed per rod. At this level, Penn & Hagins (1972) show a receptor desensitization so profound that no response is regained 10 min after the bleach. Since they used an isolated retina in which there is likely to be no regeneration of visual pigment, there is no real conflict between their results and ours.

Fig. 3 also shows an unexpectedly quick return of responsiveness in the bleached area. The recovery we measure is significantly faster than earlier reported rhodopsin regeneration rates in the rat (Tansley, 1931; Lewis, 1957; Dowling, 1963), but Perlman (1978) has reported that the time constant of regeneration varies from 2 to 50 min for bleaches which vary from weak (2–3 %) to strong ($> 60\%$). As the measurements in Tables 1 and 2 and Fig. 6 show, we measure a more rapid recovery after weak than after strong bleaches.

Threshold measures revealed a more prolonged but still rapid course of dark adaptation after small-spot bleaches. A comparison of the difference in dark adaptation after a small-spot bleach and a half-field bleach is shown in Fig. 5. When a 23 % bleach was applied as a small spot, sensitivity was regained after less than 20 min at the bleached location. When the same 23 % bleach was applied as a half-field bleach, the same test location showed an elevated threshold for more than 70 min after the bleach. This result implies that there is a spread of bleaching effects, for application of a bleach to areas adjacent to the testing locus enhances the bleaching effect. Whether this is due to bleaching signals being pooled so that bleaching can affect sensitivity in an adjacent area or from pooled scattered light is a critical question which remains completely unanswered by these experiments.

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