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Equivalence of Background and Bleaching Desensitization in Isolated Rod Photoreceptors of the Larval Tiger Salamander

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ABSTRACT Psychophysical experiments have shown an equivalence between sensitivity reduction by background light and by bleaches for the human scotopic system. We have compared the effects of backgrounds and bleaches on the light-sensitive membrane-current responses of isolated rod photoreceptors from the salamander *Ambystoma tigrinum*. The quantum catch loss was factored out from the desensitization due to bleaching to give the fraction of "extra" desensitization due to adaptation. For backgrounds, desensitization is well described by the Weber/Fechner equation. The extra desensitization after bleaches can also be described by the Weber/Fechner equation, if an "equivalent" background produced by bleaching is made linearly proportional to the fraction of pigment bleached. A background which produces an extra desensitization of a factor of two is equivalent to a fractional bleach of ~6%. Equivalent background and bleaching desensitizations were associated with similar reductions in circulating current. There is a linear relation between log flash sensitivity and decrease in circulating current. Equivalent background and bleaching desensitizations were associated with similar increases in cGMP phosphodiesterase and guanylate cyclase activity. These were inferred from membrane current changes after steps into lithium or IBMX solutions. There were also similar reductions in the integration times of dim flash responses for equivalent desensitizations produced by backgrounds and bleaches. These results suggest that the equivalence between background and bleaching found psychophysically may arise at the very earliest stages of visual processing and that these two processes of desensitization have similar underlying mechanisms.

KEY WORDS: rod photoreceptor • membrane current • light adaptation • *Ambystoma tigrinum*

INTRODUCTION

Exposing the eye to light bright enough to bleach a substantial fraction of the visual pigment produces a large decrease in sensitivity, which recovers slowly as the photopigment is regenerated. Since the decrease in sensitivity is much larger than would be expected from the decrease in the concentration of the pigment, bleaching may produce an "equivalent background" light (Crawford, 1947). This equivalent background may then produce an "extra" desensitization of the visual system much like that produced by real backgrounds (for review, see Barlow, 1972).

In single photoreceptors isolated from the retina, bleaching also produces an extra desensitization (see Fain and Cornwall, 1993). This desensitization may also arise from an equivalent background, since recent experiments with both rods and cones have shown that bleached pigment activates the transduction cascade in a manner similar to real light (Cornwall and Fain, 1994; Cornwall et al., 1995). Bleached pigment (probably as opsin) appears to do this by turning on the G protein transducin (Matthews et al., 1994), the cGMP phosphodiesterase (PDE),[1] and the guanylate cyclase, though with an efficiency (in rods) that is 10^6–10^7 times less than that for the light-excited intermediate of rhodopsin bleaching, Rh*.

If real light and equivalent light both excite photoreceptors by a similar mechanism, then both should produce similar changes in the response properties of the rods. We have tested this hypothesis by comparing the circulating current, response waveform, and PDE and cyclase velocities produced by backgrounds and bleaches in rods of the salamander *Ambystoma tigrinum*. In order to make this comparison, we have estimated the extent of visual pigment depletion after bleaching, so that the contribution of the loss in quantum catch of the bleached cells could then be factored out from the total desensitization due to bleaching, leaving only the extra desensitization to be compared with the desensitization produced by background light.

Our results failed to detect a difference in the effects of real and equivalent light on salamander rods. They also indicated that the relationship between desensitization and bleaching, which has been the subject of considerable speculation and controversy (Dowling, 1960; Rushton, 1961; Lamb, 1981; Pepperberg, 1984),

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[1] *Abbreviation used in this paper:* PDE, phosphodiesterase.

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may have a simple explanation: the desensitization may be the sum of a component due to loss of quantum catch and an extra desensitization produced by an equivalent background, whose intensity is proportional to the amount of bleached pigment, a proposal essentially the same as that used by Lamb (1981) to describe human scotopic dark adaptation. Finally, we describe a novel relationship between desensitization, by backgrounds or bleaches, and the level of circulating current.

METHODS

Rods from the larval tiger salamander (*Ambystoma tigrinum*) were isolated, and their responses were recorded as previously described (Cornwall and Fain, 1994; Jones, 1995*a*). The results presented here come from two sets of experiments. For the first set (Figs. 1–4), rods were continuously superfused with a Ringer solution which contained (in mM): 108 NaCl, 2.4 KCl, 1 $CaCl_2$, 1.2 $MgCl_2$, 1.6 NaH_2PO_4, 0.5 $NaCO_3$, 10 glucose, 10 HEPES, pH 7.8, and 100 mg/l BSA. Cells were stimulated with 10-ms, diffuse flashes from one beam of a dual-beam photostimulator, made quasi-monochromatic by passage through an interference filter (537 nm). The second beam of the photostimulator provided infrared illumination for manipulation of the cells, steady background illumination at 533 nm, or bleaching light steps at 579 nm. Light intensities were calibrated as in Jones (1995*a*). Data were recorded and analyzed with ASYST or ASYSTANT+ (Keithley Instruments Inc., Taunton, MA). In the second set of experiments (Fig. 5), rods were continuously superfused with a Ringer solution which contained (in mM): 104 NaCl, 2.5 KCl, 1 $CaCl_2$, 1.6 $MgCl_2$, 10 glucose, and 10 HEPES, adjusted to pH 7.8 with ~8 mM NaOH. In these experiments, a rapid microperfusion system was used to step rods into solutions containing Li^+ instead of Na^+ or containing 0.5 mM isobutyl-methyl-xanthine (IBMX), as previously described (Cornwall and Fain, 1994). Cells were stimulated with 20-ms, diffuse flashes, and the flashes, steady background illumination, and bleaching light steps were at 520 nm. Light intensities were fixed as in Cornwall and Fain (1994). Data were recorded and analyzed with PCLAMP (Axon Instruments, Foster City, CA).

Measurement of Flash Sensitivity

Flash sensitivity was measured as response peak amplitude divided by flash intensity (in photons μm^{-2}), for averaged responses to a series of dim flashes at the same intensity. Generally, 5–10 responses were averaged; sometimes, a larger number (up to 40). Dim flashes were taken to be flashes producing responses with peak amplitudes less than or about 10% of the maximum response amplitude.

Estimation of the Fraction of Pigment Bleached

In an isolated rod for which little or no pigment regeneration occurs, the rate of pigment bleaching during a light step is proportional to the concentration of pigment times the product of stimulus intensity and the photosensitivity of the visual pigment. The photosensitivity, in turn, is equal to the product of the absorption cross-section and quantum efficiency for bleaching (Dartnall, 1972). After a bleaching light step of duration t and intensity I_B, the fraction of pigment bleached is therefore expected to be:

$$F = 1 - \exp(-I_B \cdot P \cdot t), \quad (1)$$

where P is the photosensitivity.

For the experiments illustrated in Figs. 1–4, bleaching light steps used unattenuated light at 579 nm, lasting between 0.2 and 4.6 s. The fraction of pigment bleached was calculated from Eq. 1 assuming an in situ photosensitivity of 3.0×10^{-9} μm^2 (at 579 nm). Full details of the calculations leading to this estimate of the in situ photosensitivity will be published elsewhere (G.J. Jones, manuscript in preparation). In brief, the calculations were based on: (*a*) measurement of the average effective collecting area of salamander rod outer segments (18 μm^2 at 537 nm); (*b*) measurement of the average volume of salamander rod outer segments (1.73 pl); and (*c*) an estimate of the optical density of the pigment in situ at 579 nm as one half of the optical density at 537 nm, assuming that the visual pigment is 85% vitamin A_2-based (Hárosi, 1975). The calculations provided a value for the concentration of visual pigment in the outer segment (2.8 mM); they also took account of self-screening, the dichroic absorption of the outer segment, polarization of the light beams, and the angle of tilt between the outer segments and the plane of focus of the stimulating and bleaching beams.

Salamander rods contain predominantly a vitamin A_2-based visual pigment (Hárosi, 1975), for which the solution photosensitivity is 7.4×10^{-9} μm^2 (Dartnall, 1972). In situ, the photosensitivity will be smaller predominantly because of self screening and because of the partial alignment of the visual pigment chromophore in the plane of the disc membrane. It can be shown that the in situ photosensitivity for transverse illumination of salamander rods with peak optical density of 0.143 and a dichroic ratio of 4.0 (Hárosi, 1975) will be reduced by ~15% because of the self screening and by ~10% because of the chromophore alignment, but this reduction will be partially compensated if the outer segments contain a small fraction of vitamin A_1-based visual pigment. A crude estimate for the photosensitivity is therefore 6×10^{-9} μm^2, at a wavelength of 520 nm. This corresponds reasonably well with the estimate at 579 nm described above. A reasonable correspondence between solution photosensitivity and in situ values has previously been reported for both rods and cones: from microspectrophotometry in cones (Gupta and Williams, 1990; Jones et al., 1993) and from the early receptor current in rods and cones (Makino et al., 1991). That the photosensitivity of the visual pigment in isolated photoreceptors does not differ appreciably from that in solution implies there is little interference from photoproduct absorption during bleaching, photoreversal of bleaching, or regeneration of visual pigment. The results described here are, however, very insensitive to the precise value of photosensitivity used to calculate the fraction of pigment bleached. Repeating the data analysis with the photosensitivity increased or decreased by 15% (corresponding approximately to the situations where the chromophores are randomly or perfectly oriented) did not appreciably affect the results (see below and Figs. 2 and 4).

RESULTS

Characteristics of Bleaching Desensitization

When an isolated rod from the salamander was exposed to bright bleaching illumination, there was first a silent period during which no responses were obtained, no matter how bright the stimulus (Fig. 1 *A*). After this, flash sensitivity recovered to a steady level that was below the original level for the dark-adapted cell. At the same time the circulating current, which was zero immediately after bleaching, also recovered to a new steady level. The time courses of recovery of sensitivity

and circulating current were similar, suggesting that the two may be causally related (see below). As the intensity of the bleaching light was increased, the time course of recovery and the duration of the silent period were prolonged, but no attempt was made to investigate this relationship systematically.

In addition to the changes in circulating current and sensitivity, bleaches also produced an acceleration in the decay phase of the response (Fig. 1, *B* and *C*) and a shift of the response-intensity curve to higher intensities (Fig. 1 *D*). Some of the shift of the response-intensity curve was due to loss of quantum catch, since the light exposure in this experiment bleached about one-half of the visual pigment. However, most of the shift was due to a modulation of the transduction cascade, which has many of the characteristics of background adaptation (see Fain and Cornwall, 1993).

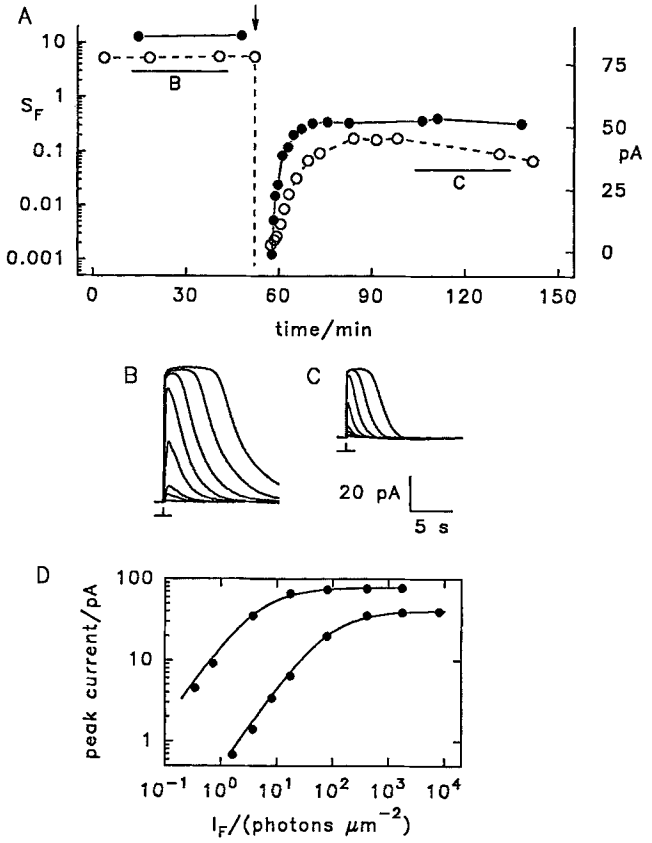

FIGURE 1. Reduction of circulating current and flash sensitivity after bleaching. (*A*) Time course of changes in circulating current, measured as the maximum light-suppressible current (○, ordinate on right) and flash sensitivity (S_F, peak pA/[photons μm^{-2}]; ●, ordinate on left). (*B–C*) Superimposed responses to flashes recorded during the times marked in *A*. Each trace is an average of 3–5 responses, bandwidth 0–5 Hz. Flash duration, 10 ms; wavelength, 537 nm. (*D*) Peak amplitudes of the responses in *B* and *C* as a function of flash photon density (I_F). Flash sensitivity was reduced 35-fold after bleaching, and the circulating current was reduced to 58% of the dark level. The fraction of pigment bleached was 0.53.

Steady-state Desensitization after Bleaching

In Fig. 2, we give the sensitivity at steady state after a bleach as a fraction of the dark-adapted sensitivity for 22 rods (14 of the data points in Fig. 2 have been taken from rods also used for other experiments; Jones, 1995*a*). Sensitivity, as a fraction of dark-adapted sensitivity, has been plotted as a function of the fraction of pigment bleached, which was calculated from the in situ photosensitivity (see METHODS). The long dashed line represents the desensitization due to quantum catch alone. It is clear that all the data points lie above this line, and the difference is an extra desensitization due to modulation of transduction. Similar results have been obtained from intracellular recordings of amphibian rods (Leibovic et al., 1987), although the extent of desensitization for a given bleach was larger in those experiments than in our suction electrode recordings.

To explain the extra desensitization not due to loss of quantum catch, we shall assume that bleached pigment excites the visual transduction cascade (Cornwall and Fain, 1994) and produces an equivalent background that adapts the rods (Barlow, 1972). We shall assume that an equivalent background adapts like a real background light.

Adaptation by Real Light

In real backgrounds, flash sensitivity in salamander rods declines according to the Weber/Fechner relationship (Matthews et al., 1988),

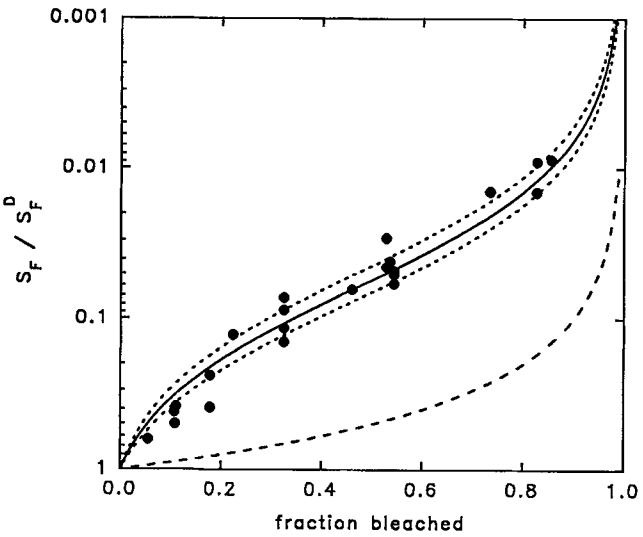

FIGURE 2. Decrease in flash sensitivity in steady state after bleaching. Each point represents a single bleach on one cell. The solid curve is Eq. 5, with k = 16.2. Short dashed curves calculated with k equal to 13.0 or 20.7, corresponding to increasing or decreasing the in situ photosensitivity by 15%. Long dashed curve indicates the loss in sensitivity expected from loss in quantum catch alone.

$$\frac{S_F}{S_F^D} = \frac{I_o}{I_o + I_B}, \quad (2)$$

where S_F is flash sensitivity, S_F^D is flash sensitivity in the dark, I_B is the background light intensity, and I_o is a constant. Eq. 2 may be re-arranged to give a derived sensitivity change that increases linearly with background light (Baylor and Hodgkin, 1974),

$$\frac{S_F^D}{S_F} - 1 = \frac{I_B}{I_o}. \quad (3)$$

In Fig. 3 *A*, we have plotted $S_F^D/S_F - 1$ as a function of I_B/I_o for 5 rods, each exposed to two or three background illuminations. Results from four of these cells have appeared previously, in a different context (Jones, 1995*a*). The parameter I_o was estimated as the mean for each rod of the values calculated from Eq. 2 for each background. The mean value across cells was 1.39 ($\pm$ 0.62, SD, $n = 5$) photons $\mu m^{-2} s^{-1}$. The line drawn in Fig. 3 *A* is Eq. 3. Agreement with Eq. 3 is reasonable, though there is some deviation at low backgrounds. This is most likely due to restricted sampling at low backgrounds, where errors are relatively high. Saturation at high backgrounds is not seen because the background intensities were not sufficiently bright. If the lowest two points of Fig. 3 *A* are excluded, a simple linear regression analysis on the data indicates that the results do not deviate from the straight line of Eq. 3 at 95% confidence limits.

Adaptation by Equivalent Light

Previous experiments with isolated salamander rods have indicated that bleached pigment molecules excite the transduction cascade in a linear fashion, such that each bleached pigment molecule acts with the same probability and gain (Cornwall and Fain, 1994). The "intensity" of the equivalent light produced by bleaching may be simply proportional to the fraction of pigment bleached. To test this notion, we have first calculated the extra desensitization (S_F'/S_F^D) from the data in Fig. 2 by removing the component due to quantum catch from the total decrease in sensitivity (S_F/S_F^D). That is, we have assumed that

$$\frac{S_F}{S_F^D} = (1 - F)\frac{S_F'}{S_F^D}, \quad (4)$$

with F calculated from Eq. 1. The results were then transformed to ($S_F^D/S_D' - 1$) as for real light. If the intensity of the equivalent light is proportional to the fraction of pigment bleached, then we might expect

$$\frac{S_F^D}{S_F'} - 1 = k \cdot F, \quad (5)$$

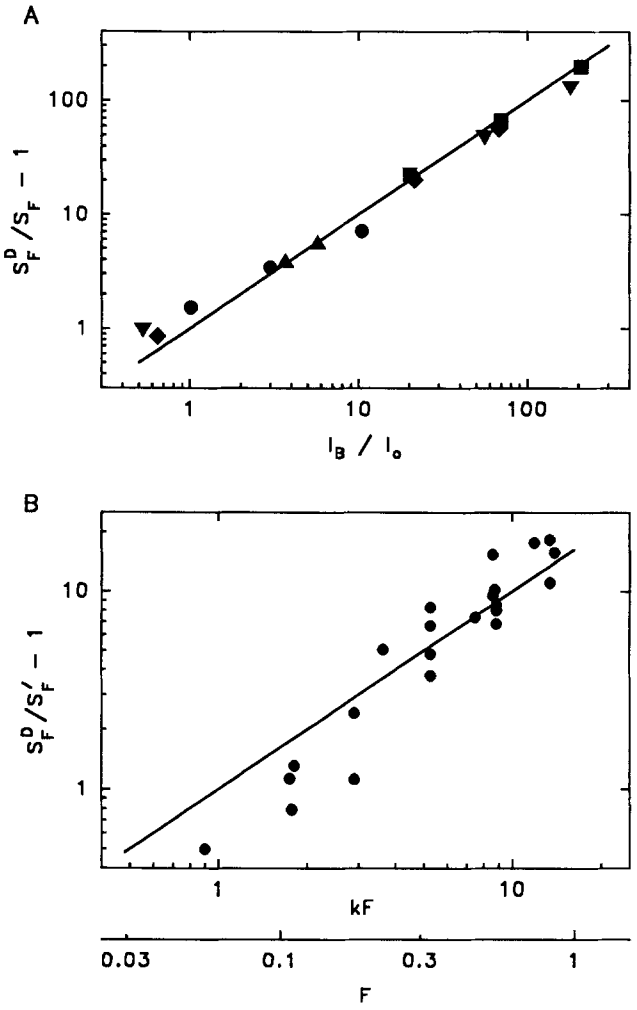

FIGURE 3. Linearized sensitivity parameter in backgrounds and after bleaching. (*A*) Background desensitization. Each symbol represents data for a single cell. The results were normalized to the mean I_o for each cell. The line is text Eq. 3. (*B*) Bleaching desensitization, after removal of the contribution from quantum catch loss. F is the fraction of pigment bleached. Data points are each from a single bleach on a single cell. The results were normalized using the mean value of k across cells. The line is text Eq. 5.

by analogy with Eq. 3. In Fig. 3 *B* we have plotted ($S_F^D/S_D' - 1$) against kF for the 22 rods of Fig. 2. Since only one value of k was available for each cell, the mean value across all cells was used. The line plotted in Fig. 3 *B* is Eq. 5. The agreement is reasonable, except at small bleaches. However, it is likely that there is a small amount of pigment regeneration after bleaching due to the isolated rods containing a small amount of *11-cis* retinal (Azuma et al., 1977; Cocozza and Ostroy, 1987), resulting in the extent of pigment bleached at steady-state being appreciably overestimated at bleaches of a few per cent. If the lowest 4 points of Fig. 3 *B* are excluded, a simple linear regression analysis indicates that the data do not deviate from the straight line of

Eq. 5 at 95% confidence limits. Furthermore, there is a substantial overlap between the 95% confidence limits of this regression and those of the data for background adaptation shown in Fig. 3 *A*.

The mean value for k was 16.2 (± 6.0, SD, $n = 22$), so that the fraction bleached which desensitizes the rod at steady-state by a factor of two is $\sim 16.2^{-1}$ or ~6%. This is equivalent to $\sim 2 \times 10^8$ opsin molecules, since the salamander rod outer segment contains $\sim 3 \times 10^9$ opsin molecules (see METHODS). This can be compared to the intensity of real light that desensitizes by a factor of two, which is about 1 photon μm^{-2} s^{-1} (see above), equivalent to ~20 excited porphyropsin molecules per second, for a collecting area of ~20 μm^2 (see METHODS).

A Model for Bleaching Desensitization

Combining Eqs. 4 and 5, we can calculate the total change in sensitivity after a bleach as

$$\frac{S_F}{S_F^D} = \frac{(1-F)}{(1+k \cdot F)}. \quad (6)$$

This equation is plotted as the solid line in Fig. 2, using the mean value of k, 16.2. A model in which bleaching desensitization is due to a combination of the loss in quantum catch and an extra component that rises linearly with the fraction of pigment bleached seems adequate to explain our data. Furthermore, this conclusion is fairly insensitive to the value taken for the in situ photosensitivity of the salamander rod visual pigment. The short dashed lines of Fig. 2 show the result of plotting Eq. 3 when the results are recalculated after increasing or decreasing the photosensitivity by 15% (see METHODS). The corresponding values of the parameter k were 13.0 and 20.7. A small deviation appears to remain after small bleaches. This is probably because, as mentioned above, there is a small, variable amount of visual pigment regeneration after bleaching in the isolated rod cell.

Relation between Sensitivity and Circulating Current

Background adaptation appears to be predominantly modulated by a Ca feedback mechanism whereby a reduction in intracellular Ca produces an increase in guanylate cyclase activity and a decrease in the activity of cGMP PDE (see Fain and Matthews, 1990; Fain and Cornwall, 1993). In turn, the internal Ca level in the rod outer segment is thought to be set directly by the level of circulating current (McNaughton, 1990). If desensitization by backgrounds and bleaching were both caused by the same Ca-dependent mechanism, then equivalent desensitizations in backgrounds or after bleaches should be associated with equivalent reductions in circulating current.

We have tested this possibility, and the results are shown in Fig. 4 *A*. The open circles show the fractional reduction in circulating current during background adaptation as a function of the flash sensitivity relative to the flash sensitivity in the dark. The results are from 23 background experiments, and include those of Fig. 3 *A*. Results from some of these cells were used previously for other analyses (data from 5 cells in Jones, 1995*a*; data from 4 cells in Pepperberg et al., 1984). The filled symbols show results from bleach-adapted cells and are from the same population of rods as in Figs. 2 and 3 *B*. The relative desensitization after bleaching is given as S_F'/S_F^D, that is the contribution from quantum catch loss has been removed. There is a close correspondence between the reduction in circulating current

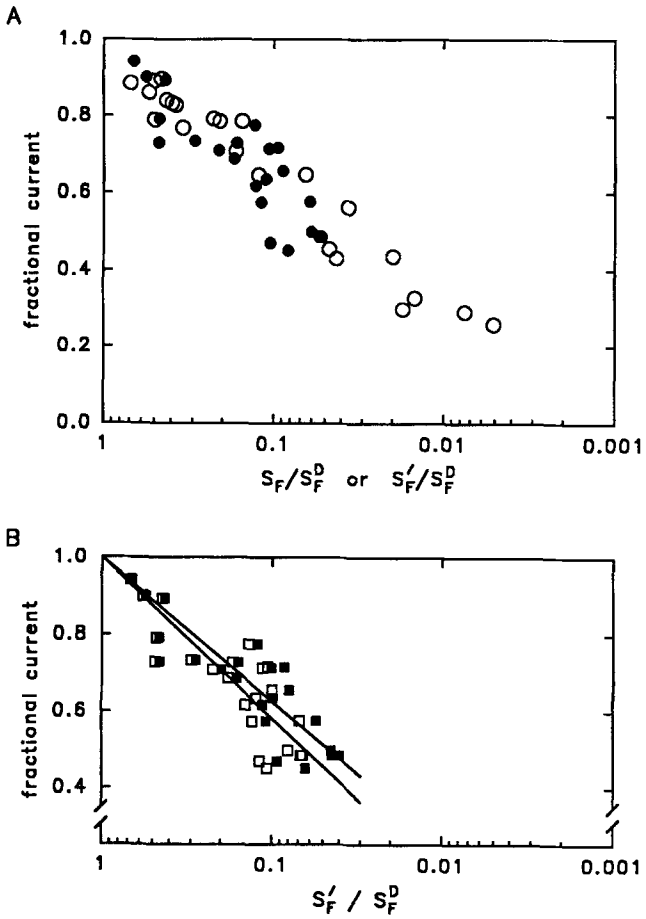

FIGURE 4. Relationship between desensitization and reduction in circulating current. (*A*) The suppression of the circulating current is shown relative to the dark circulating current and was measured as the relative reduction in the maximum light-suppressible current. ○ show the suppression of circulating current associated with desensitization due to background light. ● show results obtained in steady state after bleaching, with the contribution from quantum catch loss to the desensitization removed. See text for definitions of S_F and S_F'. (*B*) Bleaching desensitization results from *A* recalculated after increasing (■) or decreasing (□) the in situ photosensitivity by 15%. The lines are least squares fits of a straight line constrained to pass through the point (1,1).

produced by backgrounds and that produced by bleaches. Fig. 4 *B* shows that this is little affected by uncertainty in the fraction of pigment bleached, where both sets of data points for bleach-adapted cells were recalculated using a value for the in situ photosensitivity increased or decreased by 15%, together with straight lines fitted to each set. The spread of the data points in Fig. 4 *B* was comparable to that for the background-adapted cells (*open circles*) in Fig. 4 *A*.

Stimulation of PDE and Cyclase by Backgrounds and Bleaches

Previous experiments have shown that both backgrounds and bleaches accelerate the rates of the cGMP PDE and the guanylate cyclase of the rod outer segment (Hodgkin and Nunn, 1988; Cobbs, 1991; Cornwall and Fain, 1994). We have examined the possibility that equivalent changes in the rates of the PDE and cyclase are associated with equivalent changes in sensitivity and circulating current. In Fig. 5, we have plotted changes in the rates of the PDE, β/β^D, and cyclase, α'/α'^D (see Cornwall and Fain, 1994, for a full discussion of these parameters and their measurement), as a function of relative sensitivity. The data in Fig. 5 *A* were obtained from the same rods used in the study of Cornwall and Fain (1994). In Fig. 5 *B*, the original data set of Cornwall and Fain (1994) has been augmented by the addition of 7 bleach-adapted rod experiments carried out since that study was completed. The apparent changes in PDE activation and cyclase activation are here plotted against the relative decreases in sensitivity produced by backgrounds or bleaches, with the contribution from quantum catch loss removed. When plotted in this way, the data show no evidence to support the notion that these relationships are different. A similar conclusion is reached if β/β^D and α'/α'^D are plotted as functions of fractional current instead of sensitivity (not illustrated).

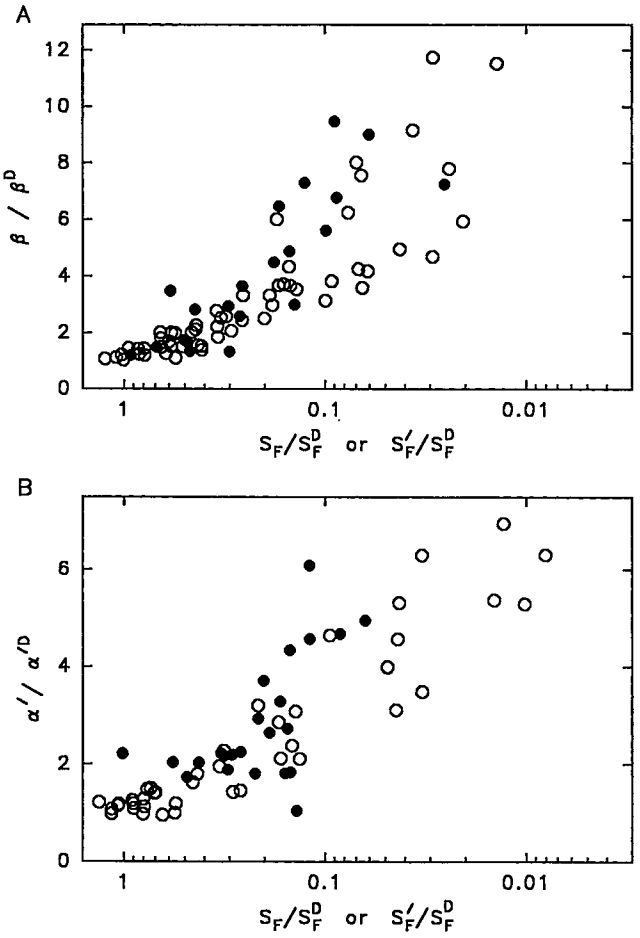

FIGURE 5. Equivalent activation of phototransduction by backgrounds and bleaches. (*A*) Activation of cGMP PDE. The parameter β was obtained from the decline in current on rapid substitution of Li^+ for Na^+ ions in the external solution. (*B*) Activation of guanylate cyclase. The parameter α′ was obtained from the current change on rapid substitution of solution containing IBMX. See text and Cornwall and Fain (1994) for details. ○ backgrounds, with sensitivity plotted as S_F/S_F^D. ● bleaches, with results obtained in steady state after bleaching and sensitivity plotted as S_F'/S_F^D.

Integration Time

The integration time, defined as the integral of the dim flash response divided by its peak amplitude, is known to decrease during light adaptation, reflecting the acceleration of the kinetics of the flash response (Baylor and Hodgkin, 1974). We have therefore used the integration time for small amplitude responses as a quantitative measure of response kinetics, in order to compare rods desensitized by backgrounds or bleaches. From a sample of 40 background conditions and 16 bleaches, we previously concluded (Fain and Cornwall, 1993) that there was no difference in the effects of backgrounds and bleaches, though the results showed considerable scatter. In the present study, we have been able to increase our sample size and compare integration times for a total of 80 background-adapted conditions and 54 bleach-adapted cells, as a function of relative desensitization (S_F/S_F^D or S_F'/S_F^D). The results with this larger sample have not been illustrated, since they are similar to those previously given. They provide no indication of any systematic difference in the response waveform between cells desensitized by backgrounds and by bleaches.

DISCUSSION

For isolated salamander rod cells desensitized by bleaching, we find that a simple model can be used to account for the extent of the desensitization as a fraction of pigment bleached. The desensitization can be explained as the sum of two components, one due to the decrease in quantum catch and the other to an equivalent back-

ground whose intensity is proportional to the concentration of bleached pigment. Such a scheme is not novel. A linear dependence of equivalent background on photoproduct concentration was originally proposed by Lamb (1981) and successfully used to model human scotopic dark adaptation curves. Subsequently, a similar scheme was obtained from analysis of bleaching desensitization in the isolated skate retina (Pepperberg, 1984), and a scheme with a quantum catch and a linear component was used to explain bleaching desensitization in isolated salamander cone photoreceptors (Jones et al., 1993). It is possible that other schemes, even a log-linear relationship (Dowling, 1960; Rushton, 1961) could be fit to our data. Nevertheless, we believe our model is plausible. In support, the velocities of both the PDE and cyclase at steady state after bleaching increase linearly with percent bleach (Cornwall and Fain, 1994), suggesting that the equivalent excitation produced by bleaching is the linear sum of the effects of bleached pigment molecules, which each stimulate the transduction cascade with the same probability and gain.

Removal of the contribution of quantum catch to bleaching desensitization enabled us to make a detailed comparison of the changes associated with desensitization by bleaching and background light. For all aspects investigated, namely the reduction in circulating current, the activation of cGMP PDE and of guanylate cyclase, and the decrease in response integration time, no difference could be detected for backgrounds and bleaches. These conclusions contradict one conclusion of an earlier study (Cornwall et al., 1990). There, it was stated ". . . the effects of bleaching and background light on σ and response compression were clearly not equivalent." However, that comparison did not completely take account of loss of quantum catch, and the amount of bleaching may well have been underestimated. There are other differences between background and bleaching adaptation that remain, such as the spread of these effects along the outer segment (Cornwall et al., 1990). It is also worth noting that adaptation by real light is accompanied by an increase in membrane current noise. A comparable increase in noise is not detected for adaptation by equivalent light after bleaching (Fain and Cornwall, 1993; Leibrock et al., 1994). Further experimental work on the membrane current noise after adaptation by bleaching and comparison with the noise produced by backgrounds is in progress (Jones, 1995*b*).

For both backgrounds (Fain and Matthews, 1990) and bleaches (Matthews et al., 1996), the change in sensitivity is thought to be mediated primarily, if not exclusively (Matthews, 1995), by a change in cytosolic Ca^{2+} concentration. The change in Ca^{2+} is thought to have several effects, including an activation of the guanylate cyclase (Koch and Stryer, 1988), a modulation of the lifetime of photo-excited rhodopsin (Kawamura, 1993), a decrease in the activation of the PDE (Lagnado and Baylor, 1994), and an increase in the open probability of the light-dependent channel (Hsu and Molday, 1993). The relative importance of these effects for backgrounds has recently been investigated (Koutalos et al., 1995). It may be different for bleaches. Nevertheless, it would seem that the mechanisms of desensitization for backgrounds and bleaches are similar.

Although the mechanisms seem similar, they may not be identical. It is possible, for example, that the steady current response of the rod (see Fig. 4) is altered in backgrounds by a Ca^{2+}-dependent modulation of the lifetime of photo-excited rhodopsin (Kawamura, 1993); a similar mechanism may not be important after bleaches (see Cornwall and Fain, 1994). Subtle differences in the mechanisms may be difficult to detect, because the range over which sensitivity is altered is different for the two kinds of adaptation. Since bleached pigment is so much less efficient than Rh* in stimulating the transduction cascade (Cornwall and Fain, 1994), even a 99% bleach modulates the cascade less than a moderately bright background. For this reason, it is possible that the mechanisms for background and bleaching adaptation seem similar only because some processes (e.g., cyclase modulation) are more important for dim backgrounds than for bright ones (Koutalos et al., 1995). Additional experiments will be required to resolve these uncertainties.

Though subtle differences may exist in mechanism, our experiments and those of others (Leibovic et al., 1987) show that the similarity of the effects of backgrounds and bleaches is great enough to account, at least in part, for the psychophysical equivalence of the two forms of adaptation. We have examined adaptation only over a limited range and only in the photoreceptors. Furthermore, we have looked at the effects of bleaches only at steady state, in the absence of visual pigment regeneration. Nevertheless, we believe that the equivalence we have observed is likely to be at least a component of dark adaptation under physiological conditions. This seems especially likely after large bleaches, for which considerable desensitization would be caused by opsin. The roles of other pigment intermediates and other cells in the retina remain a subject for further study.

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