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Responses of crayfish photoreceptor cells following intense light adaptation

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Summary. After intense orange adapting exposures that convert 80% of the rhodopsin in the eye to metarhodopsin, rhabdoms become covered with accessory pigment and appear to lose some microvillar order. Only after a delay of hours or even days is the metarhodopsin replaced by rhodopsin (Cronin and Goldsmith 1984). After 24 h of dark adaptation, when there has been little recovery of visual pigment, the photoreceptor cells have normal resting potentials and input resistances, and the reversal potential of the light response is 10–15 mV (inside positive), unchanged from controls. The log V vs log I curve is shifted about 0.6 log units to the right on the energy axis, quantitatively consistent with the decrease in the probability of quantum catch expected from the lowered concentration of rhodopsin in the rhabdoms. Furthermore, at 24 h the photoreceptors exhibit a broader spectral sensitivity than controls, which is also expected from accumulations of metarhodopsin in the rhabdoms. In three other respects, however, the transduction process appears to be light adapted: (i) The voltage responses are more phasic than those of control photoreceptors. (ii) The relatively larger effect (compared to controls) of low extracellular Ca^{++} (1 mmol/l EGTA) in potentiating the photoresponses suggests that the photoreceptors may have elevated levels of free cytoplasmic Ca^{++}. (iii) The saturating depolarization is only about 30% as large as the maximal receptor potentials of contralateral, dark controls, and by that measure the log V-log I curve is shifted downward by 0.54 log units. The gain (change in conductance per absorbed photon) therefore appears to have been diminished.

Introduction

Photoreceptors lose sensitivity in the light and regain sensitivity in darkness. There are several underlying processes, which are different in detail in vertebrate and invertebrate eyes. In both vertebrates (Dowling 1963) and invertebrates (Brown and Coles 1979) relatively rapid changes in sensitivity occur at intensities that photoconvert negligible quantities of rhodopsin. In the ventral photoreceptor of *Limulus*, early stages of sensory adaptation involve an increase in the concentration of intracellular calcium (Brown and Blinks 1974; Lisman and Brown 1975a, b; Harary and Brown 1984). In invertebrates, higher intensities of light convert rhodopsin to metarhodopsin, forming a thermally stable photosteady-state mixture of the two pigment forms, and threshold rises in direct proportion to the decreased probability of absorption by rhodopsin (Seldin et al. 1972; Fein and DeVoe 1973; Hamdorf and Rosner 1973; Hamdorf et al. 1973; Bruno et al. 1977; Lisman and Strong 1979). Metarhodopsin therefore normally exerts neither an excitatory nor an inhibitory effect on light-adapted receptors.

When the eyes of living crayfish are exposed for about 30 s to orange adapting lights that drive the visual pigment throughout the eye to a photosteady state, the pigment composition in the rhabdom is about 20% rhodopsin and 80% metarhodopsin (Cronin and Goldsmith 1982). Some hours after the adapting exposure, rhabdoms sampled from the eyes have a distinctly altered morphology, in which the bands of microvilli have become less evident and the surfaces of the organelles are covered with granules of accessory screening pigment (Cronin and Goldsmith 1984). Transmission elec-

tron microscopy (Tostanoski and Goldsmith, unpublished observations) indicates that the loss of microvillar order that is apparent in the light microscope is caused by extensive vesiculation of individual microvilli as well as penetration of the rhabdoms by intrusions of cytoplasm.

Dark recovery of both rhodopsin and the normal morphology in these intensely adapted photoreceptors requires several days, and in some animals more than a week. Although the process appears to be continuous once started, with the total concentration of photopigment (rhodopsin plus metarhodopsin) in each rhabdom remaining constant within the uncertainty of measurement, some rhabdoms delay the onset of recovery. As a result, midway through the process most of the rhabdoms comprise two morphological classes: those that have recovered their normal banded and relatively clean appearance, and others with a disordered aspect and more granules of secondary screening pigment adhering to their surfaces. Some are intermediate in appearance. In general, those photoreceptor organelles that look disordered and pigmented have a high content of metarhodopsin, whereas those that have recovered their normal shape contain mostly rhodopsin. Based on this correlation, Cronin and Goldsmith (1984) have hypothesized that the mechanism of recovery of rhodopsin involves new synthesis of rhabdomeric membrane.

After one day in the dark following the intense adapting exposure, these animals provide an opportunity to study photoreceptor sensitivity in cells containing high titers of metarhodopsin but at a time when other processes of adaptation have normally had ample time to complete. We find that in this experimental system, persistence of metarhodopsin and the presence of membrane disorder is accompanied by a decreased capacity of the cell to generate photocurrent that is characteristic of light adaptation.

Materials and methods

Animals. Specimens of the crayfish *Procambarus clarkii* were obtained from Carolina Biological Supply. They were exposed to overhead fluorescent lights on a 12-h light:12 h dark cycle in aerated freshwater aquaria and fed dog food.

Adapting exposures. In order to bring all of the rhodopsin in the eye into photosteady state with metarhodopsin, the eyes of living animals from which the antennae had been removed were placed at the focus of a parabolic mirror and irradiated for 30 s with intense orange light (Corning 3–66 long-pass filter, 50% transmission at 570 nm) or blue light (Corning 5–56, peak transmission at 420 nm, 200 nm band width at half peak) (Cronin and Goldsmith 1984). Quantum flux at the cornea was about 10^{18} photons s^{-1} cm^{-2}. With each animal, one eye was irradiated and the other used as an unirradiated control. After these irradiating exposures the animals were returned to darkness until used for further examination.

Electrical recording. Animals were selected for experiments by recording from the unirradiated control eye. If the receptor cells in the control eye had average intracellular responses of at least 10 mV to a standard test flash then the animal was used to complete the experiment. This physiological screening was done in order to select the healthiest animals for study, and about half of the animals in the laboratory population were rejected. This number fluctuated with the time of year, and the proportion of acceptable animals was higher in summer than in winter.

For electrophysiological recording the eyestalk was excised and all structures distal to the retinal layer were dissected away under dim red light. The eye was pinned to a cork base in a lucite chamber filled with physiological saline (van Harreveld 1936). Intracellular recording electrodes had resistances of about 40–60 MΩ when filled with 3 mol/l KCl and immersed in crayfish saline. The reference electrode was a chlorided silver wire in a glass capillary tube filled with 3 mol/l KCl in 2% agar. The preparation was dark-adapted for at least an hour after being mounted in the recording chamber. All recordings were from the main rhabdom, i.e. retinular cells 1–7.

Optical stimulator. For spectral sensitivity measurements light (10 nm half band width) from a grating monochromator (Bausch and Lomb, 250 mm focal length) and a 500 W tungsten-iodide lamp was focused onto the eye using a combinaton of glass lenses and fiber optics. Intensity was controlled with a pair of counter rotating quartz-inconel optical wedges, and an electromagnetic shutter delivered flashes of light of 100 ms duration. The wedges and light source were calibrated with a photodiode (United Detector Technology) of known sensitivity. Spectral sensitivity was measured by stimulating the eye with a series of narrow wavelength bands (10 nm half band width) 20 nm apart. Stimuli were separated by dark intervals of up to a minute. The wedges were adjusted for a criterion depolarization at each wavelength, and the spectrum was swept in both directions in order to catch any systematic drift in sensitivity of the cell. The narrow wavelength bands had insufficient intensity to measure the V-log I curves of strongly adapted cells, and for this purpose the monochromator was removed and the eye stimulated with white light at intervals of up to 1 min.

Results

Spectral sensitivity

Figure 1 A shows average spectral sensitivities of retinular cells at 24, 48, and 72 h after exposure to the intense orange light. Note that even after 24 h of dark adaptation the cells appear to be about 0.8 log units less sensitive than control receptors in the unirradiated eye and that full recovery is slow, requiring several days. Second, the spectral sensitivity functions are broadened at 24 h compared to the controls.

Changes in sensitivity following intense blue light are shown in Fig. 1 B. The results are similar to those observed following orange light, except

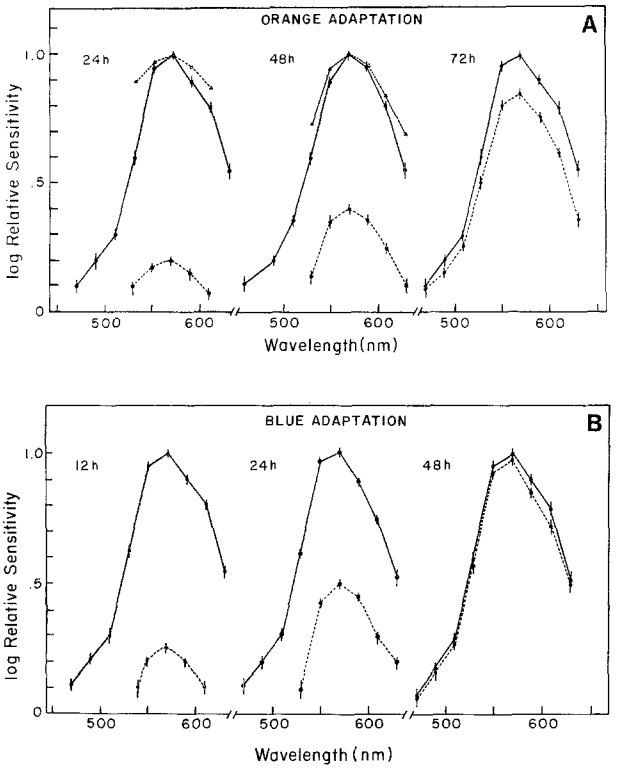

Fig. 1. **A** Spectral sensitivity curves of crayfish retinular cells (R1–R7), based on intracellular measurements made after 24, 48, and 72 h of dark adaptation following 30-s irradiation with an intense orange light. For each time the solid curve (above) is an average of 5 cells from the control eye, and the lower, dashed curve describes the sensitivity of 5 cells from the exposed eye. For the data at 24 and 48 h, the curve for the experimental cells has been translated vertically and replotted (open triangles) to show more clearly the broadening effect caused by the screening of rhodopsin by metarhodopsin. Error bars indicate ±1 standard error of the mean. **B** Recovery of sensitivity at 12, 24, and 48 h following exposure to an intense blue light. Otherwise as described for **A**

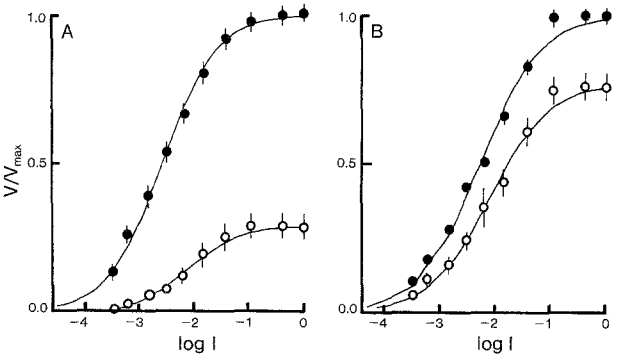

Fig. 2. **A** V-log I curves for receptors 24 h after irradiation with intense orange light (open circles), compared with unirradiated cells in the contralateral eye (filled circles). Each data set is the average of 9 cells; error bars indicate ±1 s.e.m. The data were obtained from two animals (4 or 5 cells in each eye). Curves through the experimental points were drawn according to Eq. (2) (with $\alpha = 0.9$), and with the experimental data scaled to the average value of V_{max} for the unirradiated controls. **B** Similar to **A** but recorded 48 h after the orange light. Data points for the control and irradiated receptors represent averages of 10 and 9 cells respectively. Exponent in Eq. (2) is 0.8

that the depression in sensitivity observed at 24 h is not as great and recovery is virtually complete after 48 h. Likewise, Cronin and Goldsmith (1984) observed that presence of metarhodopsin and loss of membrane order were less persistent following irradiation with intense blue light.

V-log I functions

In the experiments to be described below, we have limited our attention to the more severe effects of the orange light, with particular emphasis on the state of the cells at 24 h after irradiation. Under these conditions the pigment of the rhabdoms is typically 75–80% metarhodopsin and a minority of the rhabdoms show gross morphological signs of recovery (Cronin and Goldsmith 1984).

The responsiveness of individual cells was characterized further by measuring the size of the depolarization as a function of the log of the stimulating flash (white light) at various times during recovery. The filled circles in Fig. 2A show the average V-log I function based on 9 individual cells from two control eyes, and the open circles are average results on 9 cells in the two contralateral eyes, measured after 24 h of dark adaptation following irradiation with orange light and scaled to the maximum responses of the control cells. The control data are reasonably well fit by the function

$$V/V_{max} = I/(I+\sigma), \quad (1)$$

where $\sigma = I$ at $V = V_{max}/2$ (Naka and Rushton 1966). Slightly better fit is achieved by a modified form of this expression (Boynton and Whitten 1970):

$$V/V_{max} = \beta I^{\alpha}/(I^{\alpha}+\sigma^{\alpha}) \quad (2)$$

with the exponent $\alpha = 0.9$ (and $\beta = 1$, $\log \sigma = -2.6$), and this is what is shown in Fig. 2A. The curve through the experimental data (open symbols) is the same function, but with $\beta = 0.29$ and $\log \sigma = -2.1$. In other words, twenty-four hours after the intense orange light, the maximal responses of the experimental cells are only 29% as large as the saturating responses of control receptors. This response compression is described by the parameter β. At the same time, the half-saturation point (σ) of the V-log I curve is shifted to higher intensity. Note that two parameters β and σ are necessary to characterize the changes in responsiveness of the adapted cells; because of the response compres-

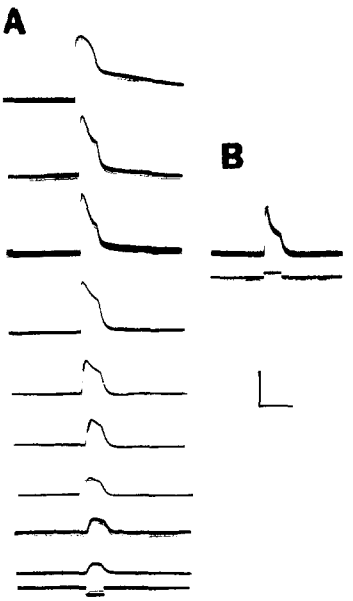

Fig. 3. A Depolarizing photoresponses of a control cell to a series of flashes of bright white light (log I=0 in Fig. 2) of 500 ms duration delivered at 15 s intervals. Note the depolarizing afterpotential in the first response of the series. As the cell light adapts, the response becomes more phasic and ultimately diminishes in size. Calibration marks 20 mV, 1 s. **B** Response of another cell after 24 h dark adaptation following exposure in vivo to an intense orange light. Test flash as in **A**. The rapid decay of the initial depolarization, creating a transient, and the absence of a long-lived depolarizing afterpotential are characteristic of a partially light-adapted cell

sion of adapted receptors, the apparent difference in sensitivity of experimental and control cells assessed from the energy required to elicit a response of constant amplitude, as in Fig. 1, will depend on the magnitude of the criterion response that is selected.

Figure 2B shows average V-log I responses at 48 h (open symbols) compared with control cells from the contralateral eyes (filled symbols). The experimental curves show significant recovery relative to the data at 24 h; for the curves $\alpha=0.8$, $\beta=0.78$, and the difference in σ is smaller.

Resting potential. When adaptation of receptors is measured by superimposing a test flash on a background light, the V-log I curves exhibit varying degrees of response compression, caused in part by depolarization of the cell. In order to see whether adapted cells of crayfish might give smaller maximal responses than controls because of a long-lasting depolarization, the resting potentials of receptors 24 h after an intense orange exposure were compared with unexposed controls in the contralateral eye. There was no difference; in this experiment, the orange-adapted photoreceptors had resting potentials of -30 ± 3.3 mV (±1 s.e., $n=10$) and the controls, -28 ± 2.0 mV ($n=10$).

Shape of the voltage response

Figure 3A shows a series of responses of an initially dark-adapted control cell to flashes of white light at the highest intensity available. This was at log I=0 on the abscissa of Fig. 2 and was of saturating intensity. The test flashes were delivered at 15 s intervals and caused the cell to become progressively more light-adapted. Figure 3B shows a single typical response of an experimental cell to the same test flash. This cell had been exposed (*in vivo*) for 30 s to the intense orange light the previous day, and the animal kept in the dark for the intervening 24 h. The rapid decay of the initial depolarization, creating a transient, and the absence of a long-lived depolarizing afterpotential are characteristic of a normal cell that is partially light-adapted (compare with the second and third responses in A).

Effect of low external Ca^{++}

The more phasic character of the voltage responses of experimental cells suggested that even after 24 h of dark adaptation they might have elevated levels of internal Ca^{++}, and that it might be possible to supply some relief by bathing them in Ca^{++}-free saline. The effect of a Ca^{++}-free saline containing 1 mmol/l of the calcium sequestering agent EGTA on control and experimental cells is shown in Fig. 4. As expected, low $[Ca^{++}]$ in the bathing medium increased the duration of the voltage response of the cells to light, slowed the decay of the transient, and increased the overall amplitude (Fig. 4A). The effects of low Ca^{++} were proportionally larger in the cells that had been irradiated with orange light on the previous day (Fig. 4B), but even in low Ca^{++} the responses of experimental photoreceptors to saturating stimuli were still much smaller than the maximal responses of control cells in normal saline. Moreover, a series of test flashes of maximal intensity caused additional light adaptation of experimental cells bathed in EGTA (Fig. 4C).

Reversal potential

A large response compression would be observed if the reversal potential of the light response were to become negative as a result of the prior irradiation with orange light. This possibility was tested by measuring the reversal potential of control and

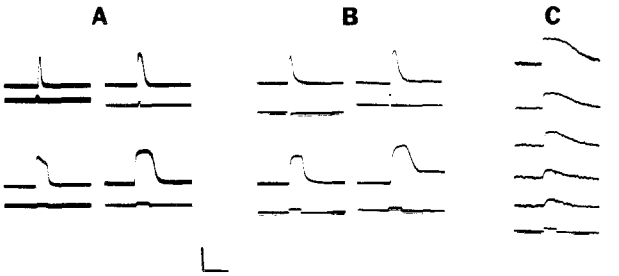

Fig. 4A–C. Effects of low Ca^{++} on a control cell (**A**) and on a photoreceptor that was exposed on the previous day to an intense orange light and then kept in the dark for 24 h (**B**). Left column (**A** and **B**), cell in control saline; right column, Ca^{++}-free saline containing 1 mmol/l EGTA. Upper row (**A** and **B**), voltage responses to 100 ms test flashes; bottom row, to 500 ms flashes. The responses are larger and last longer in low external calcium; note the proportionally greater effect in **B** as compared to **A**. Calibration marks 10 mV in **A**, 5 mV in **B**; time mark 1 s in both. **C** Photoresponses of an experimental cell to a series of intense white flashes (log I = 0 in Fig. 2). The cell had been exposed in vivo 24 h earlier to the orange isomerizing light and then dark-adapted 24 h. The cell was placed in low-calcium saline (10 mmol/l EGTA) for recording, and test flashes were given at 15 s intervals. Every second response is shown. Calibration marks 20 mV, 1 s

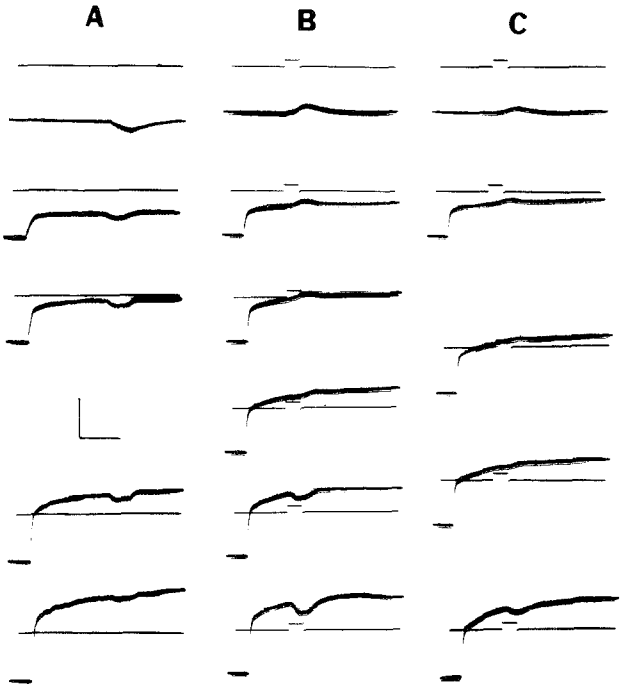

Fig. 5A–C. Reversal potential of the photoresponse is little affected 24 h after irradiation with orange light. Membrane voltage was set by passing current through the recording electrode, using a bridge circuit as described in the text. **A** A control series in which extrinsic currents depolarized the membrane to various extents; the current was zero in the top record and increased from the top to the bottom of the figure. The trace that is initially uppermost in each record (monitor trace in **B** and **C**) is positioned at 0 mV on the voltage axis of the response trace. In each record the cell received a small hyperpolarizing pulse of current of 100 ms duration superimposed on the depolarizing current. Depolarization caused a decrease in input resistance, but the bridge remained in balance with all values of depolarizing current used. **B** and **C** Light responses arising from different values of membrane voltage. Membrane potential was depolarized with current as in **A**, and the light monitor trace is positioned at 0 mV on the voltage axis of the response trace. **B** control receptor. **C** receptor 24 h after irradiation with orange light. In both cells the reversal potential of the photoresponse was +10 to +15 mV. Calibration marks 250 ms, 50 mV. These cells had larger resting potentials than some of the cells studied in other experiments

experimental cells (Fig. 5). For this experiment a single intracellular electrode was employed, along with a bridge circuit to balance the voltage drop in the electrode caused by the current that was used to set the resting membrane voltage. Figure 5A shows a control series in which a small hyperpolarizing pulse of current was superimposed on the larger depolarizing current that was used to set the membrane voltage. At the resting potential the membrane time constant was clearly longer than the 100 ms hyperpolarizing test pulse. As the membrane was depolarized, the input resistance of the cell decreased, but the bridge remained in balance despite the current through the electrode.

Figure 5B shows the photoresponses of a control cell when the membrane potential had been depolarized to various extents with an extrinsic current, and Fig. 5C shows a corresponding series of responses measured 24 h after orange light. In both cells the reversal potential of the light response was positive. Average values were 14 ± 3.0 mV ($\pm$s.e., $n = 8$) for the control cells and 12 ± 1.6 mV ($n = 5$) for the experimental photoreceptors. These results indicate that 24 h after orange light the reversal potential of the light response is essentially normal.

Input resistance

If the passive conductance of the membrane were to increase as a result of the orange light, a given photocurrent would produce a smaller change in membrane potential. The input resistance of control and experimental cells, as measured from the resting potential with the bridge and small hyperpolarizing pulses of current, was 9.0 ± 1.32 MΩ ($\pm$s.e.m, $n = 17$) and 9.3 ± 2.11 MΩ ($n = 16$), respectively.

Discussion

Analysis of the response-energy function – effects of light adaptation and metarhodopsin

The underlying causes of the long-term loss of sensitivity of the orange-irradiated photoreceptors are

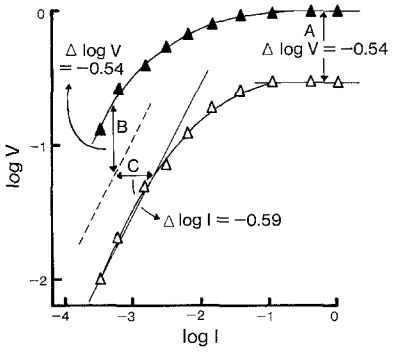

Fig. 6. Data of Fig. 2A replotted on log-log axes. Twenty-four hours after orange light the response-energy functions of individual cells are displaced downward 0.54 log units due to a decrease in gain. This is evident when saturating responses are compared (arrow A). Below saturation, however, the responses of orange-adapted cells are smaller than expected from this decrease in gain (arrow B) because the response-energy curves also appear to be translated to the right by 0.6 log units due to loss of rhodopsin (arrow C)

easier to identify when the response-energy data of Fig. 2A are replotted as log V vs. log I (Fig. 6). The light adaptation that occurs at such low adapting fluxes that there is no significant loss of rhodopsin might be expected to shift the log V-log I curve downward. That is, the gain should change, and the amount of conductance change per absorbed photon should be less than in the dark-adapted state. This exact relationship has been inferred for human photoreceptor signals by psychophysical criteria (Alpern et al. 1970a, b, c). On the other hand, direct measurements of adaptation of *Limulus* ventral photoreceptors to background lights give a somewhat different result. Under voltage clamp, background illumination causes a large change in gain in the linear region of the response-energy function, but relatively little response compression (Brown and Coles 1979). Our measurements differ from both of these studies in that our test flashes were not superimposed on background adapting fields.

The change in the response-energy function that is attributable to the decreased concentration of rhodopsin can be calculated more readily. When a substantial fraction of the visual pigment has been converted to metarhodopsin and the cell allowed to dark adapt, the log V-log I curve should be shifted to the right by an amount that reflects the decreased probability of absorption by rhodopsin. The shape of the curve should remain unaltered.

The voltage responses of photoreceptors 24 h after the orange light saturate at about 30% of the saturating depolarization of unirradiated controls; by this criterion the log V-log I curve thus appears to be shifted downward 0.54 log units (Fig. 6, double-headed arrow A). Three features of this response compression indicate that it is due to light adaptation of the transduction process and is not a secondary phenomenon: i) the reversal potential of the light response is unchanged (Fig. 5), ii) the resting potentials of the photoreceptors have recovered to the same value as dark controls, and iii) there is no difference in the input resistance of dark and orange-treated receptors. In terms of the shape of the voltage response (see Figs. 3–4 and the section below on the possible role of calcium) the photoresponses also appear to be light-adapted.

If the log V-log I curve of the orange-irradiated cells were simply displaced downward by 0.54 log units, the lower limb would fall in the position indicated in Fig. 6 by arrow B. The data points, however, are an additional 0.6 log units to the right (double-headed arrow C). The fraction of the visual pigment remaining as rhodopsin after the eyes are irradiated with the orange light is 0.21 (0.16–0.28) (Cronin and Goldsmith 1984). On the assumption that this produces a fall in sensitivity that is determined by the decreased probability that incident energy will be absorbed by rhodopsin, the calculated lateral shift of the log V-log I function along the log I axis is 0.67 (0.55–0.8) log units, in reasonable agreement with the observed value of 0.6 log units (Fig. 6, double-headed arrow C). Thus the log V-log I function for the orange irradiated cells appears to be displaced downward by 0.54 log units due to a lingering change in gain and to the right by 0.6 log units due to the loss of rhodopsin.

The effects on spectral sensitivity of large amounts of metarhodopsin homogeneously intermingled with rhodopsin throughout the volume of the rhabdom have been quantitatively modeled for the crayfish (Goldsmith 1978). The primary effect of screening by metarhodopsin should be to broaden the spectral sensitivity function, but this prediction has not been tested heretofore. The spectral sensitivities of photoreceptors measured after bright orange light are significantly broader than those of controls, both at 24 h and at 48 h (Fig. 1A). A similar effect is present in the curves measured after blue adaptation, but at comparable times it is smaller.

Relative rates of recovery following orange and blue lights

The relative rates of recovery of sensitivity after irradiation by blue and orange lights parallel the

different times over which rhodopsin reappears in the rhabdoms: recovery of sensitivity and regeneration of rhodopsin are both faster after blue adaptation than after orange. Likewise, the larger variation in the absolute sensitivities of individual receptors from adapted eyes compared to controls (note error bars in Fig. 2A) has its counterpart in the variation in rhodopsin content of receptors during the time course of pigment regeneration. Blue light may enhance the supply of 11-*cis*-retinal (Cronin and Goldsmith 1984).

Possible role of Ca^{++}

As described above, several observations point to a persistent light adaptation that involves more than the presence of metarhodopsin. In general, light adaptation of arthropod photoreceptors makes the responses more phasic. In the ventral photoreceptor of *Limulus*, the mechanism has been shown to involve an increase in free intracellular Ca^{++}, either from the extracellular solution or by release from internal sequestering sites (Brown and Blinks 1974; Lisman and Brown 1975b; Harary and Brown 1984). As internal calcium rises, the response decays from transient to plateau, and there is a decrease in the photocurrent per photon absorbed (Lisman and Brown 1975b). In crayfish (Stieve and Hanani 1976), low external Ca^{++} (EGTA) increases the duration of the ERG and speeds dark adaptation, consistent with the more detailed information available for *Limulus*.

The photoresponses of orange-adapted crayfish cells do not have the properties of fully dark-adapted receptors, even after 24 h dark adaptation. Their decreased ability to maintain depolarizations indicates that they may have elevated levels of intracellular calcium, and this hypothesis is indirectly supported by the effects of low Ca^{++} in the external solution in increasing the amplitude of the light response and increasing its duration. Both of these effects are larger in the orange-irradiated cells than in dark controls (Fig. 4A–B). Delay in recovering normal levels of internal calcium therefore appears to be one possible effect of the orange light. On the other hand, lowering the external Ca^{++} does not produce a three-fold increase in the amplitude of the voltage response; consequently a persistent Ca^{++} load is unlikely to be responsible for the major part of the response compression that is observed in the experimental photoreceptors at 24 h (Fig. 2A). Some other factor in the transduction process is also involved (see also Levy and Fein 1985).

Possible consequences of membrane disorder

The total amount of pigment (rhodopsin + metarhodopsin) seems to be the same in control cells and in receptors 24 h after irradiation with orange light (Cronin and Goldsmith 1984). This suggests that the experimental cells have a normal amount of rhabdomeric membrane, although the cytological observations show that it is less well organized. If some of this pigment-containing membrane were no longer incorporated into the cell surface it would contribute to the spectrofluorometric measurements of metarhodopsin but not to the photoresponse measured with an intracellular electrode. Removal of surface membrane would decrease the maximum photocurrent that the cell is capable of generating, but it could also be accompanied by an increase in input resistance. Furthermore, disorganization of the rhabdom might be accompanied by a loss of intercellular coupling, which is known to occur between some of the retinular cells of crayfish ommatidia (Muller 1966), and a loss of coupling should also cause an increase in the average input resistance. The measurements of input resistance show no change, however, and further work will be required to clarify this point.

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