Retinal Mechanisms of Visual Adaptation in the Skate

DANIEL G. GREEN, JOHN E. DOWLING, IRWIN M. SIEGEL, and HARRIS RIPPS

From the Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138, the Marine Biological Laboratory, Woods Hole, Massachusetts 02543, and the Departments of Ophthalmology and Physiology, New York University School of Medicine, New York 10016. Dr. Green's present address is the Vision Research Laboratory, University of Michigan, Ann Arbor, Michigan 48104.

ABSTRACT Electrical potentials were recorded from different levels within the skate retina. Comparing the adaptive properties of the various responses revealed that the isolated receptor potential and the S-potential always exhibited similar changes in sensitivity, and that the *b*-wave and ganglion-cell thresholds acted in concert. However, the two sets of responses behaved differently under certain conditions. For example, a dimly illuminated background that had no measurable effect on the sensitivities of either of the distal responses, raised significantly the thresholds of both the *b*-wave and the ganglion cell responses. In addition, the rate of recovery during the early, "neural" phase of dark adaptation was significantly faster for the receptor and S-potentials than for the *b*-wave or ganglion cell discharge. These results indicate that there is an adaptive ("network") mechanism in the retina which can influence significantly *b*-wave and ganglion cell activity and which behaves independently of the receptors and horizontal cells. We conclude that visual adaptation in the skate retina is regulated by a combination of receptoral and network mechanisms.

INTRODUCTION

Recent electrophysiological studies have emphasized the importance of the photoreceptors in the process of visual adaptation (Boynton and Whitten, 1970; Dowling and Ripps, 1971, 1972; Grabowski et al., 1972; Witkovsky et al., 1973; Normann and Werblin, 1974). For example, incremental thresholds of rods and cones can be raised more than a thousandfold by background fields that bleach only trivial fractions of the visual pigments, and such sensitivity changes appear to be reflected in the responses of more proximal elements (Dowling and Ripps, 1971; Werblin, 1974). In addition, it has been known for some time that after intense light adaptation visual sensitivity

recovers in parallel with the regeneration of visual pigment in the receptors (Dowling, 1960, 1963; Rushton, 1961, 1965 *a*; Dowling and Ripps, 1970).

On the other hand, there is no doubt that other adaptive mechanisms also participate in the determination of retinal sensitivity. For example, the threshold of a localized retinal region may be influenced by illumination falling outside the test area, and the evidence is compelling that the effect is mediated primarily by retinal interaction rather than by stray light (Kaplan and Ripps, 1960; Rushton, 1965 *b*; Easter, 1968; Werblin, 1974). It is not at all certain, however, at which stage of signal processing such adaptive mechanisms operate, or which cells are responsible for setting the level of retinal sensitivity that is reflected in the signals transmitted to higher visual centers.

The present experiments, involving the recording of electrical responses arising from different levels within the skate retina, were designed to distinguish between the receptoral and nonreceptoral components of adaptation, and to assess the relative contributions of each to steady-state adaptation and the kinetics of dark adaptation. The skate retina is ideally suited for such studies since it possesses only one type of visual receptor (rods), and it thus affords the opportunity to examine a single photosensitive system over its entire operating range (Dowling and Ripps, 1970).

METHODS

Experiments were performed on small pieces of eyecup (~1 cm square), excised under dim red light from the tapetal region of the dark-adapted skate (*Raja erinacea* or *R. oscellata*). The tissue was housed in an electrically shielded enclosure and maintained under a stream of moist oxygen as described previously (Dowling and Ripps, 1970). Three types of recording were made: (*a*) low impedance, chlorided-silver electrodes placed on the sclera and vitreous, respectively, were used to record either the gross electroretinogram from the normal preparation, or the mass receptor potential after bathing the eyecup in a solution containing 50 mM sodium aspartate (Dowling and Ripps, 1970, 1972); (*b*) glass micropipettes, filled with 2 M KCl and having an input resistance of 30–50 MΩ, were employed for intracellular measurements of horizontal cell activity (S-potentials) (Dowling and Ripps, 1971); and (*c*) Woods metal-filled glass pipettes (with tip diameters of 1–5 μm) or conventional capillary micropipettes filled with elasmobranch Ringers were used to obtain extracellular recordings of the discharge of individual ganglion cells.

Photic stimuli were delivered by a dual-beam optical system (Dowling and Ripps, 1971) that provided independent control of intensity, duration, and spectral composition of the test and adapting fields. For all the experiments described in this paper, the stimulus fields were slightly larger than the piece of eyecup and uniformly illuminated the overlying retina. Test and adapting fields were "white" lights of equal intensity derived from tungsten-iodide lamps filtered by a BG 38 heat-absorbing glass. The effective quantal content of the fields was estimated by adjusting the

intensity of the white stimulus so as to give the same electrical response (*b*-wave) as that produced by a 500-nm stimulus. The quantal irradiance on the retina for the unattenuated 500-nm stimulus, measured with a calibrated photocell, was 3.1×10^5 quanta incident/μm^2/s. Since 1.5 log units of attenuation was required to produce the same physiological effect, the unattenuated white stimulus (test or adapting field) delivered the equivalent of 9.3×10^6 quanta (500 nm)/μm^2/s. Throughout this paper, intensity scales give the logarithmic attenuation of the light beam produced by a neutral density filter of the designated value (e.g. $\log I = -5$).

Animals were dark adapted for at least 3 h before the start of an experiment. After the preparation was placed in the recording chamber and aligned with the stimulus field, it was left in complete darkness for an additional 30 min in order to ensure that the retina had reached its dark-adapted state. Incremental thresholds were measured upon backgrounds of increasing intensity (in steps of 0.5 log unit), in each instance after a stable threshold had been attained. At low intensities, this required about 4 min of light adaptation; with high intensity backgrounds, it was often necessary to wait 15–20 min before determining threshold. The interval between test flashes was sufficient so as not to disturb the prevailing state of adaptation.

RESULTS

In the experiments described here, it is necessary to compare the adaptive properties of responses that are very different in character; they originate at different levels within the retina, have different waveforms, and are obtained with different recording methods. One requirement, therefore, is to select for each response a criterion change (the index of threshold) that is appropriate to the nature of the response and its magnitude. It is impossible, for example, to obtain identical responses from a spike-generating ganglion cell and an amplitude-modulated horizontal cell. Nevertheless, threshold can be defined as the stimulus intensity that elicits a just-detectable signal. Table I lists the response criteria that gave reliable threshold measurements for each class of potential recorded in this study and typical values of the relative intensities (I_D) required to generate criterion responses in the dark-adapted preparation. The same criteria were used to assess the changes in threshold (ΔI) induced by the parameters of adaptation, and the different

TABLE I

RESPONSE CRITERIA

Electrical response	Threshold criterion	Log I_D
Receptor potential	5 μV	−7.5
S-potential	1–2 mV	−8.3
b-wave	10 μV	−9.8
Ganglion-cell discharge	1–2 spike potentials	−10.7

responses were compared by plotting for each the ΔI values relative to its own absolute (i.e., dark-adapted) threshold.

Light Adaptation

Fig. 1 shows the increment threshold data for the isolated receptor potential and the S-potential, responses that have very different amplitudes and ab-

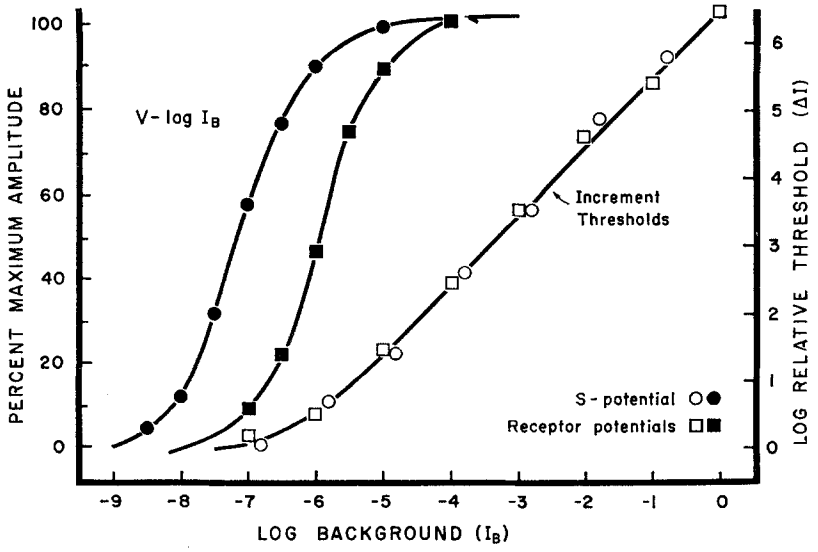

FIGURE 1. A comparison of isolated receptor potential (squares) and S-potential (circles) voltage vs. intensity (V-log I) relationships measured in the dark-adapted eye using the background light as the stimulus (filled symbols), and increment thresholds measured against background illumination (I_B) (open symbols). The S-potential V-log I curve is shifted about 1 log unit to the left on the intensity axis, indicating that the intracellularly recorded S-potential responds to lower light levels than the grossly recorded receptor potential. However, when absolute thresholds are equated and increment thresholds plotted, both responses show similar changes in sensitivity over 6 log units of background luminance. In these and in all subsequent experiments, the duration of the test flash was 0.2 s. These data are averages from three to five experiments.

solute sensitivities, but whose thresholds are affected equally by ambient illumination. In the left-hand side of the figure are the voltage vs. intensity (V-log I) curves for the S-potential and the receptoral response, each graphed relative to its own voltage maximum ($\sim$40 mV and 150 μV, respectively). The separation of the two curves on the scale of intensities indicates that the S-potential is about 1 log unit more sensitive than the receptor potential, due probably to its greater integrative capacity, i.e. the ability to pool signals from large numbers of receptors as well as from neighboring horizontal cells (Kolb, 1970; Kaneko, 1971). But in spite of the 10-fold difference in sensi-

tivity and 800-fold difference in recorded amplitude, the two potentials exhibit the same threshold variation in response to changes in background illumination (I_B). As shown in the right-hand side of the figure, the incremental thresholds for both rise together with a slope of one over a range of I_B covering 6 log units. Note particularly that even at levels of I_B which can elicit large S-potentials (e.g. log I_B = −7), the change in threshold is negligible; only when I_B is sufficient to depress receptoral sensitivity is there a desensitizing effect upon the S-unit. Clearly then if spatial summation enhances the sensitivity of S-units, it does not affect significantly the light-adapting properties of these elements. The S-potential incremental threshold appears to be determined solely by the sensitivity of the photoreceptors.

A strikingly different situation is obtained when the increment-threshold results for the *b*-wave and the receptoral response are compared (Fig. 2). As in the previous figure, the data have been graphed relative to the absolute

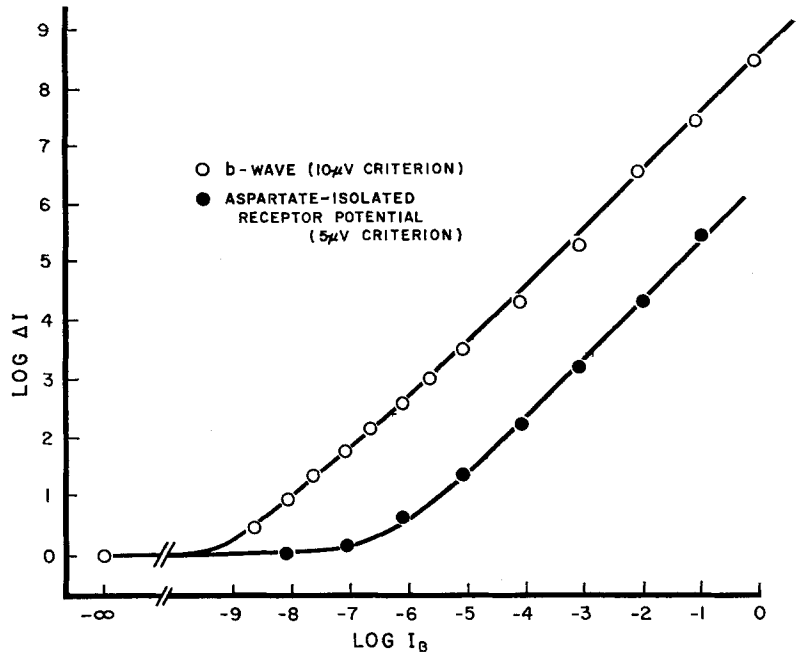

FIGURE 2. Representative increment thresholds for the *b*-wave and isolated receptor potential. The *b*-wave data were obtained from one eye of an animal; the isolated receptor potential data from the other eye. As in Fig. 1, the absolute thresholds for both responses have been equated at log ΔI = 0. Thresholds were determined 4 min or more after each increase in I_B to ensure that a stable threshold level had been reached. With dim backgrounds (I_B = −9 to −7) the *b*-wave threshold is significantly altered but there is no discernible effect on receptor threshold. With brighter backgrounds, the sensitivity of the receptor response is also decreased and both functions rise in parallel with a slope of about one.

thresholds equated at log $\Delta I = 0$. It is immediately apparent that there is a range of background intensities which have no discernible effect on receptoral thresholds, but which produce a large increase in *b*-wave threshold. For log $I_B = -7$ to -9, the receptor potential maintains its dark-adapted sensitivity, whereas *b*-wave sensitivity decreases by about 2.0 log units. We term the loss of *b*-wave sensitivity to dim backgrounds *network adaptation*, because it does not appear to be of receptoral origin. With more intense backgrounds, on the other hand, the sensitivity of the receptoral response is also depressed, and both curves rise in parallel along the so-called Weber-line (i.e. $\Delta I = I_B \times$ constant). Although it is more difficult to identify the factors controlling sensitivity over this portion of the graph (see Discussion), the important point is that background levels above log $I_B = -7$ cause the *b*-wave and receptor potential sensitivities to decrease in parallel.

As noted above, the choice of response criteria is arbitrary, and the stimuli that are used in the determination of thresholds vary widely from one type of response to the next. It is appropriate, therefore, to examine the possibility that these factors are responsible for the observation that dim backgrounds depress the sensitivity of the *b*-wave while having no apparent effect on the receptoral response or the S-potential. The results shown in Fig. 3 indicate that this is unlikely. The effects of a steady background field (log $I_B = -8$) are to shift the V-log I curve of the *b*-wave about 1 log unit to the right on the scale of abscissae and to reduce concomitantly the maximum amplitude of the response. On the other hand, the corresponding data for the receptor potential and the S-potential are not at all changed by this level of ambient illumination. Consequently, even if a 575-μV criterion had been chosen for the *b*-wave threshold and e.g. a 5-μV criterion for the receptor potential, criteria which would have equated both absolute thresholds at log $I_t = -7.4$, the background field still would have produced a decrease in *b*-wave sensitivity of 1 log unit (cf. Fig. 2).

The results presented so far suggest the presence of an adaptive mechanism located within the retina that is independent of changes in the sensitivity of the photoreceptors or horizontal cells. Although the *b*-wave is probably a glial potential (cf. Faber, 1969; Miller and Dowling, 1970), the cells underlying its generation appear to be located in the inner nuclear layer (Brown, 1968, Faber, 1969). There is, therefore, a question as to whether such steady-state adaptation is modified by events occurring *proximal* to the inner nuclear layer. Fig. 4 compares the incremental threshold data for the *b*-wave with the responses of four "on"-center ganglion cells. The absolute sensitivities of the latter were about 1 log unit greater than that of the *b*-wave (Table I), but when this difference is allowed for by equating dark-adapted thresholds, the data of Fig. 4 show that the decreases in ganglion cell sensitivity due to I_B are almost exactly the same as those obtained from *b*-wave measurements.

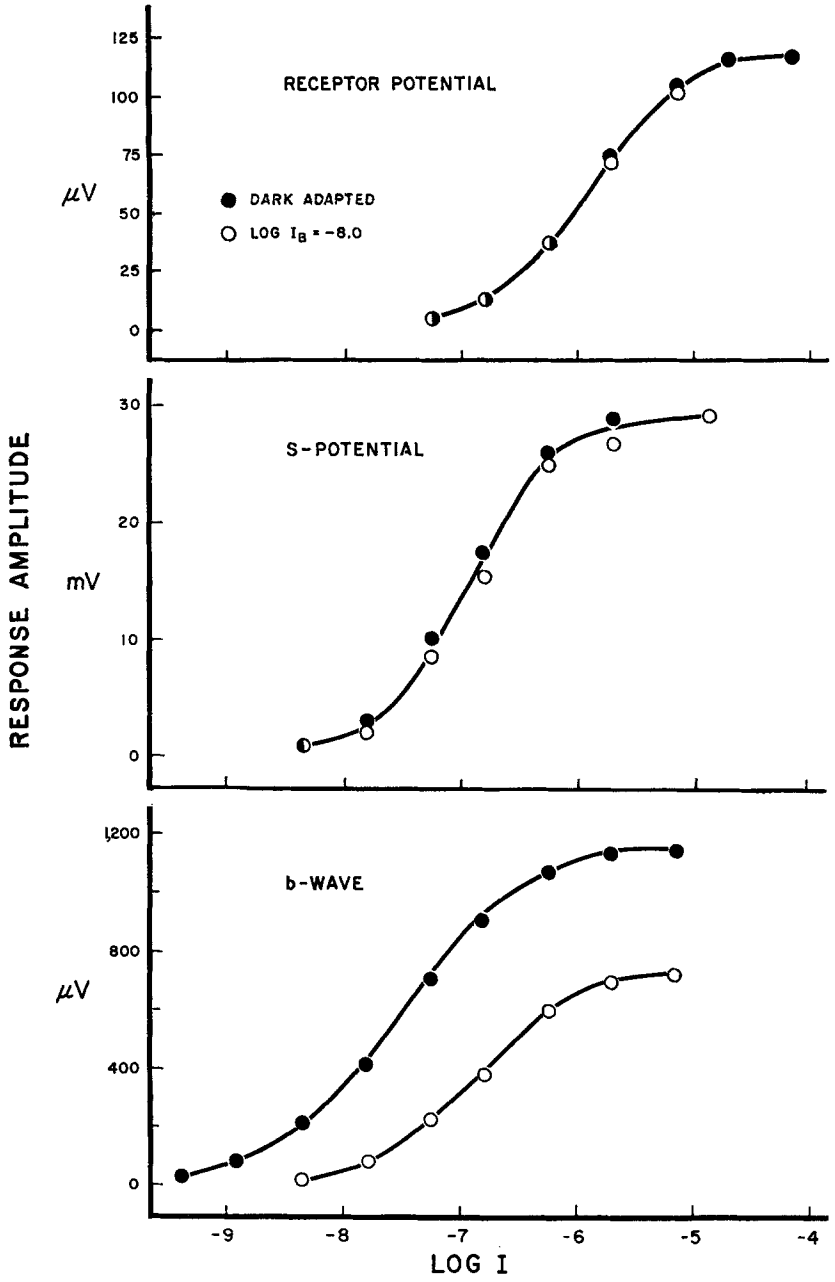

FIGURE 3. Intensity-response (V-log-I) curves for the receptor potential, S-potential, and b-wave recorded in the dark and in the presence of a dim adapting field (log I_B = −8). The V-log I curve of the b-wave is shifted by about 1 log unit to the right by this background field and its maximum amplitude is reduced. However the receptor potential and S-potential are not affected by this level of illumination. Data from representative single experiments.

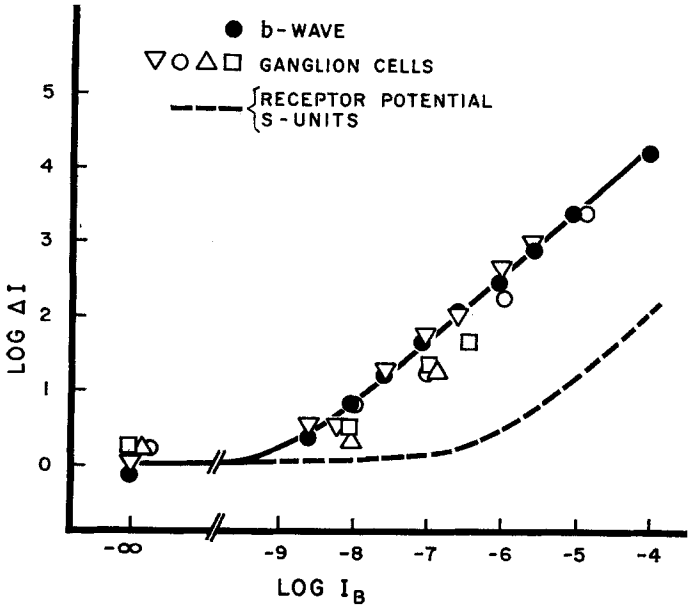

FIGURE 4. Increment threshold functions for four ganglion cells (open symbols) and *b*-wave (filled circles). The dark-adapted ganglion cell thresholds were adjusted by 1 log unit to approximate the dark-adapted *b*-wave threshold. The alterations in increment thresholds as a function of background intensity were identical for both responses and clearly different from the increment threshold curve for the receptors and S-potential response (dotted line).

These findings confirm and extend the results of an earlier study (cf. Fig. 10 of Dowling and Ripps, 1970), and again illustrate the degree to which *b*-wave sensitivity in the skate reflects the sensitivity of the retinal ganglion cells. It should be noted that transient adaptive effects occurring at the onset of illumination have been shown to originate in the inner plexiform layer and to result in transient alterations in ganglion-cell sensitivity (Werblin and Copenhagen, 1974). These effects were not the concern of the present experiments; the data of Fig. 4 show only the steady-state threshold changes that are brought about by maintained background illumination.

As shown in Fig. 3, the sensitivity (and amplitude) of the *b*-wave are markedly depressed by ambient illumination so weak that it has virtually no effect on the response characteristics of the S-potential. It would seem, therefore, that network adaptation does not involve the horizontal cells. Nevertheless, it is conceivable that a highly nonlinear relationship exists between the horizontal-cell membrane potential and the magnitude of its adapting effect; indeed, nonlinear feedback from the horizontal cells onto receptors and bipolar cells has been proposed as the basis of adaptation in the cat (Enroth-Cugell and Shapley, 1973). However, this mechanism does not seem

to be utilized by the skate retina as illustrated by the following experiment (Fig. 5).

The simultaneous recordings of the S-potential and *b*-wave shown in Fig. 5 illustrate how each type of response reacts to a weak adapting field; the test flashes were of constant intensity throughout the experimental run. With the onset of I_B, the membrane potential of the S-unit rapidly hyperpolarized to a level (~ -15 mV) that was maintained for the entire period of light adaptation. In addition, the incremental responses, although reduced

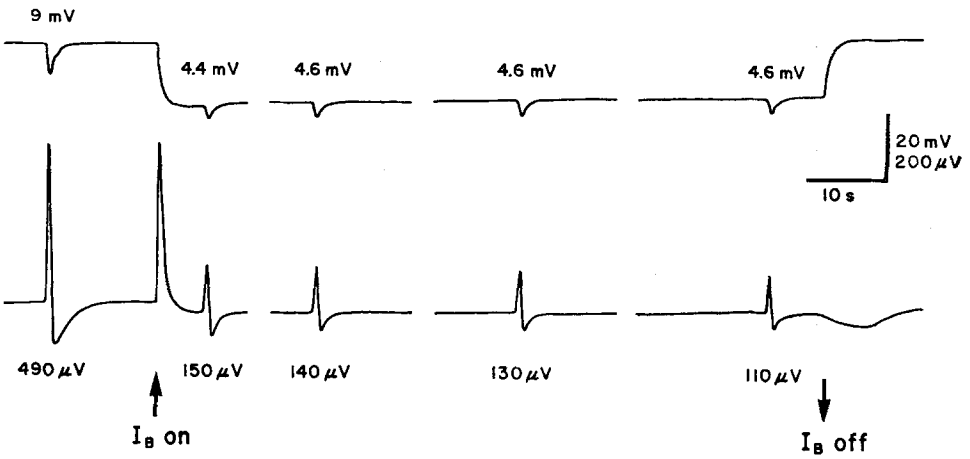

FIGURE 5. The effect of a dim background field (log $I_B = -7.2$) on intracellular measurements of the membrane potential and response amplitude of an S-unit (upper trace), and the transretinally recorded *b*-wave (lower trace); the intensity of the test flash was fixed at log $I_t = -7.2$. The total period of light adaptation was about 2 min and responses were obtained at ~15-s intervals (the duration represented by breaks in the traces). The maximum response amplitude of the S-unit was 27 mV.

in size, very quickly reached a constant amplitude so that within 20 s both the membrane potential and the response amplitude had stabilized. Now if the horizontal cell mediates the adaptive effects observed proximal to it, the way in which it responds to background illumination should be reflected to some extent in the *b*-wave responses. But the *b*-wave behaved quite differently. Immediately after the onset of I_B, there was a large decrement in response magnitude, and the responses continued to decrease in amplitude during the period of light adaptation. In fact, it often took more than 5 min before responses of constant amplitude were observed, and depending upon the level of I_B, the change in amplitude could be of either sign; i.e., with brighter backgrounds the responses grew in size during light adaptation (cf. Dowling and Ripps, 1970). This unusual feature is reflected also in the measurements of *b*-wave thresholds during light adaptation. As shown in Fig. 6,

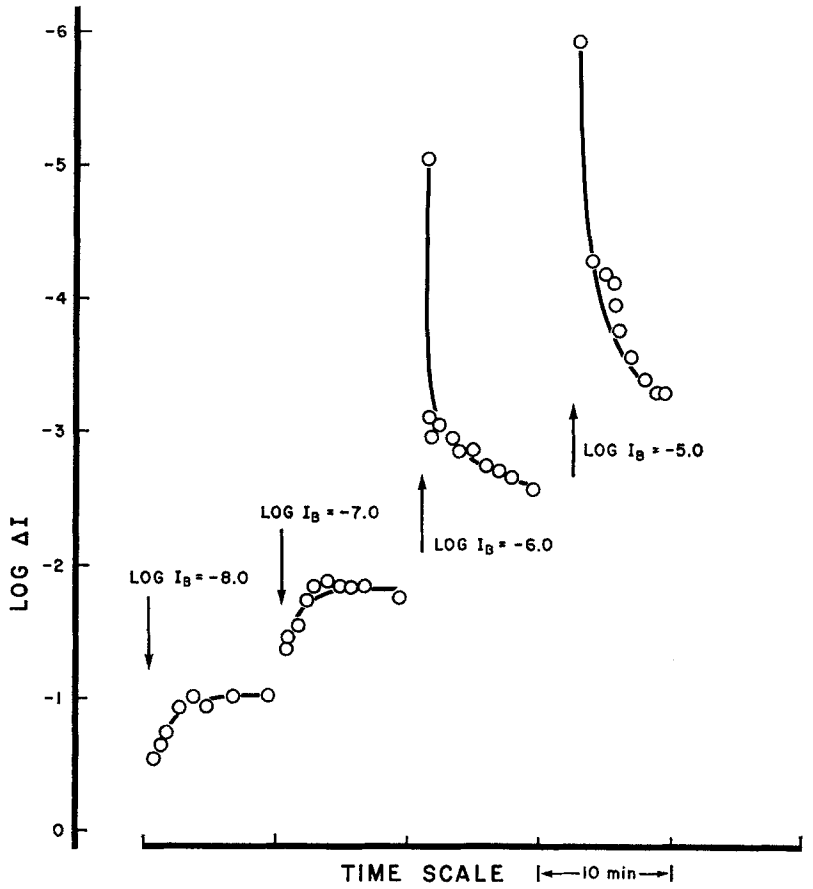

FIGURE 6. The temporal course of light adaptation to background fields of various intensities. *b*-wave thresholds relative to the dark-adapted level (log ΔI = 0) are plotted as a function of the time after the onset of each adapting field (arrows). The data, from a single experiment, are displaced arbitrarily along the time scale for clarity of presentation.

thresholds measured after the onset of very weak adapting fields (I_B = −8, −7) gradually *rise* to a stable value (plateau); with backgrounds that initially saturate the *b*-wave response (I_B > −6; cf. Fig. 3), thresholds *fall* to the light-adapted plateau. Thus, there is no apparent correlation between any of the adaptive properties exhibited by the *b*-wave and the changes in either the response magnitude or the membrane potential of the S-unit.

The previous data suggest that the rate at which the proximal retina light adapts is governed by a network mechanism that proceeds independently of the horizontal cells. Similarly, the kinetics of the receptor potential are not rate limiting. In Fig. 7 the light-adapting properties of the receptor potential

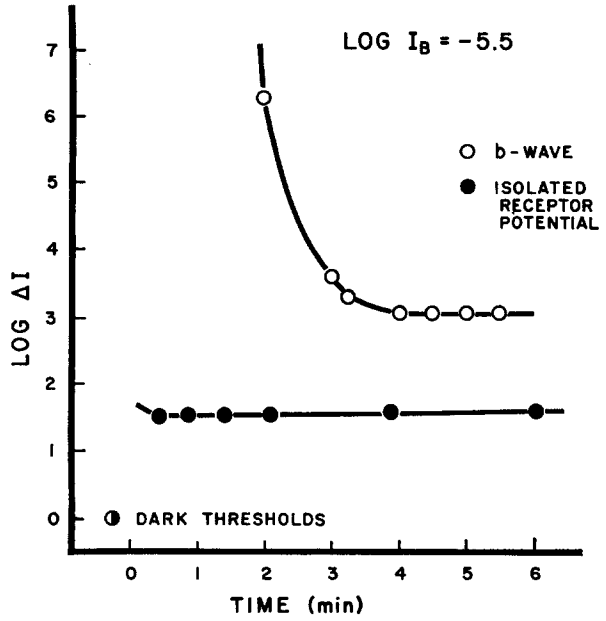

FIGURE 7. Comparison of the time-course of light adaptation (log I_B = −5.5) for the receptor potential and the *b*-wave. Incremental thresholds are graphed as a function of time after the onset of I_B. The receptor potential adapted within 30 s; the *b*-wave was unresponsive for the first 2 min, and 4 min elapsed before a steady state was reached. Data from representative single experiments.

and the *b*-wave are compared directly. The intensity of the background field (I_B = −5.5) fell just below the saturation level of the receptor potential, but above that of the *b*-wave (cf. Fig. 3). Consequently, receptoral thresholds could be determined almost immediately after the onset of I_B, and as shown by the filled symbols of Fig. 7 the plateau of light adaptation was reached quickly (<30 s). For the *b*-wave, however, an incremental response could not be elicited for nearly 2 min no matter how intense the stimulus. And when the retina again became responsive, it took an additional 2 min for thresholds to decline to a stable value. Thus, the temporal changes in the *b*-wave during light adaptation appear to be controlled by a relatively slow process that is associated with the network itself.

Dark Adaptation

Our experiments on the temporal course of dark adaptation gave further evidence of an adaptive mechanism in the distal retina that is not linked to the sensitivity changes of either the photoreceptors or the horizontal cells. Fig. 8 shows dark-adaptation data for the various electrical potentials studied; each set of results was obtained after a 1-min exposure to a weak adapting

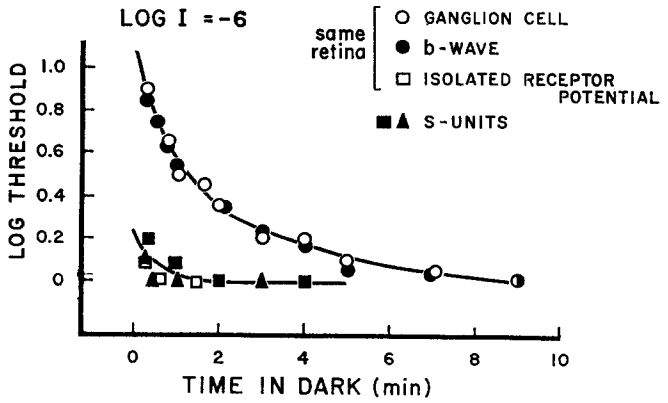

FIGURE 8. Dark adaptation of receptor potential, *b*-wave, ganglion cell, and S-potential after a 1-min exposure to log $I_B = -6$. The ganglion cell response, *b*-wave, and isolated receptor potential were measured on the same retina, the latter after the application of sodium aspartate. Threshold data are plotted relative to the dark-adapted threshold for each type of response. The ganglion cell and *b*-wave follow the same time-course of recovery and take about 8 min to fully dark adapt. The receptor potential and the S-potential recover full sensitivity within 1 min of the offset of the adapting stimulus.

field that bleached less than 0.1% of the available rhodopsin. There is again the same dichotomy as observed in light adaptation: the sensitivity of the horizontal cell recovers in parallel with that of the receptors, whereas ganglion cell sensitivity follows the course of the *b*-wave. Furthermore, it took only about 1 min for the receptor response and S-potentials to regain dark-adapted thresholds, while the *b*-wave and ganglion-cell discharge required 8–10 min to recover fully.

Owing to the very rapid dark adaptation exhibited by the receptoral response and the S-potential (Fig. 8), it is difficult to decide whether the two rates are in fact the same. However, Fig. 9 shows that after an adapting exposure 1,000 times more intense, the recovery of S-unit sensitivity is still virtually identical to that of the receptor potential over the entire 15-min period of dark adaptation.

Another experiment, shown in Fig. 10, demonstrates that in dark adaptation (as in light adaptation) the network operates independently of horizontal-cell activity. The simultaneous recordings of S-potentials and *b*-waves were obtained before, during, and after light adaptation, and again show that the *b*-wave responses are in no way correlated with changes in either the membrane potential or the response amplitude of the S-unit. The membrane potential of the latter returned to its dark-adapted level within 30 s after extinguishing the background, and the amplitude of the S-potential evoked by a constant-intensity flash reached its dark-adapted voltage in less than 2

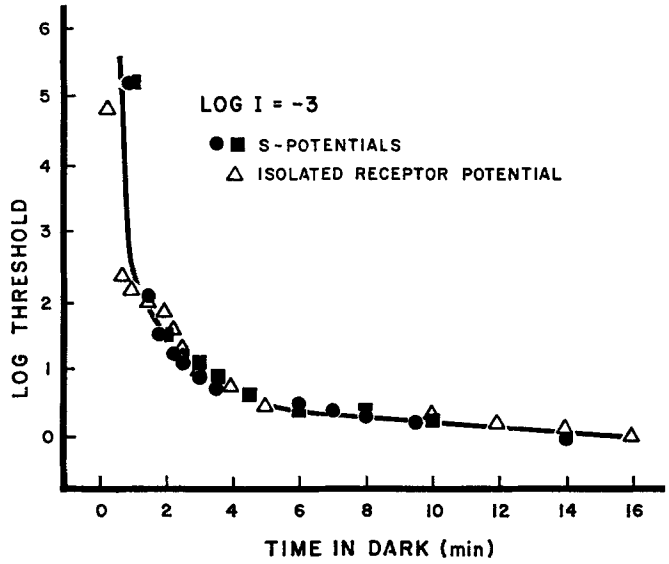

FIGURE 9. Comparison of the dark-adaptation curves for the S-potential and isolated receptor potential after exposure to a background of log $I_B = -3$. The two responses recover sensitivity with the same time-course, taking 8 min to recover fully. Data from representative single experimental runs.

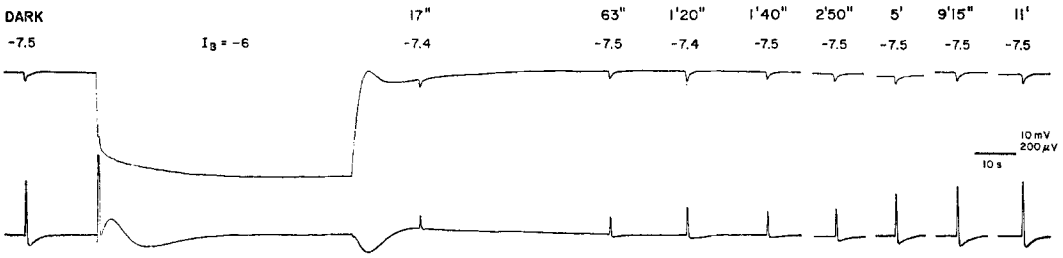

FIGURE 10. The effects of dim light adaptation on subsequent dark adaptation of the S-potential (upper traces) and *b*-wave (lower traces). Intracellular responses of the S-unit and transretinal *b*-waves were recorded simultaneously before, and after a 1-min exposure to log $I_B = -6$. Within 20–30 s after extinguishing the adapting field, the S-unit membrane potential had returned to its dark-adapted level, and within 1½ min the S-potential had regained full sensitivity. The *b*-wave, on the other hand, required about 10 min to fully dark adapt.

min. But after 2 min of dark adaptation, the *b*-wave was only 50% of control amplitude, and thereafter, while S-unit activity remained unchanged, the *b*-wave continued to increase in amplitude over the next 8–10 min of dark adaptation.

Fig. 11 illustrates another curious feature of network adaptation, namely that under certain conditions the subsequent course of dark adaptation

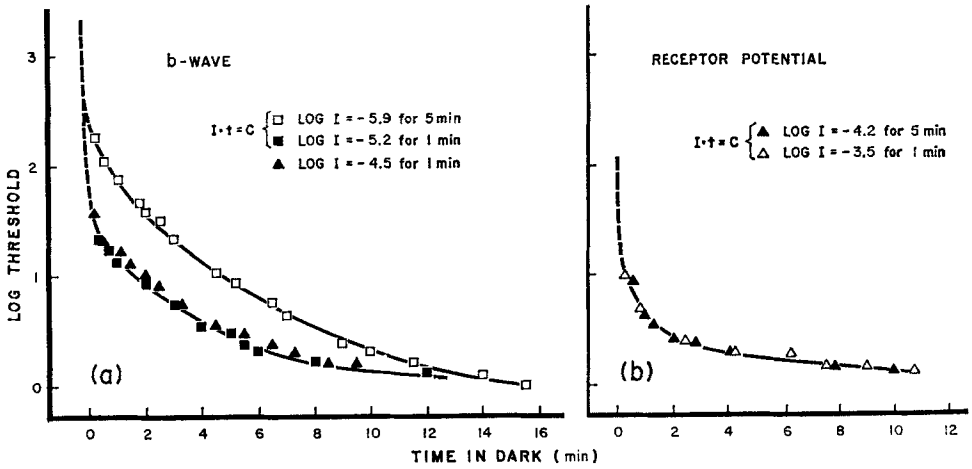

FIGURE 11. The effect of exposure duration on dark-adaptation curves for the *b*-wave as a function of time in dark after preadapting exposures of equal energy but different durations (squares), and vice versa (filled symbols). (*b*) Receptoral thresholds for equal energy exposures of different durations. Data from representative single experiments.

depends more on the duration of the preadapting exposure than on either the rate at which quanta are delivered or the total quantal content of the exposure. Thus, when two different periods of light adaptation are equated in terms of energy ($I \cdot t$), the longer exposure, provided that it exceeds 30 s, produces a slower rate of recovery. In Fig. 11 *a*, the filled squares show dark adaptation of the *b*-wave after a 1-min exposure to log $I = 5.2$; the empty squares are the results after a 5 times longer exposure at one-fifth the intensity. After the stronger but shorter exposure, the initial fall in threshold was more rapid and the eventual return to dark-adapted levels occurred earlier than after the weaker, longer exposure.

The peculiar influence of exposure duration on *b*-wave dark adaptation was observed also in a corollary experiment in which measurements were obtained after a 1-min exposure to a more intense adapting field (filled triangles). Although the higher intensity field (log $I = -4.5$) delivered quanta at five times the rate of the dimmer field (log $I = -5.2$), the dark-adaptation curves were nearly identical.

We have measured dark adaptation for the receptor potential under comparable experimental conditions, but with very different results. The data of Fig. 11 *b* show that the temporal course of dark adaptation depends largely on the total number of quanta delivered to the eye; i.e., when the product $I \cdot t$ is the same, the receptor potential dark adapts along the same time-course. In fact, in a few other experimental runs (not shown), recovery was slightly faster after the longer light-adapting exposure.

It should be emphasized that the dark adaptometry described above was

obtained after photic exposures that bleached trivial fractions of the rhodopsin content of the skate retina (cf. Dowling and Ripps, 1970). The effects of more intense light adaptation on the dark-adaptation curves of the *b*-wave and the receptor potential are shown in Fig. 12. Results for the brightest field (log $I = -1$), which bleached more than 90% of the visual pigment show that in these circumstances recovery proceeds in two phases: there is a rapid fall in threshold during the first 10–15 min in darkness, followed by a slower component that lasts about 2 h. The former is unrelated to changes in

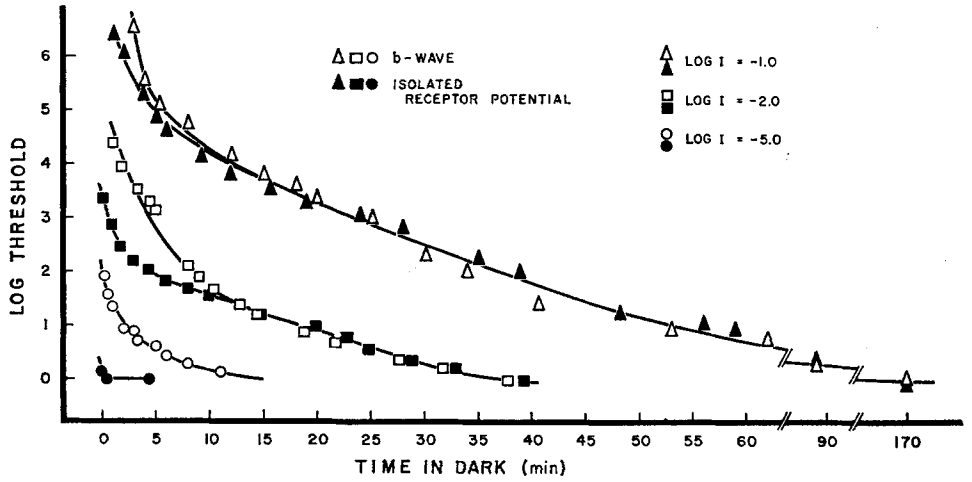

FIGURE 12. Receptoral and *b*-wave thresholds during the course of dark adaptation after 5-min preadapting exposures of various intensities. The two higher intensities, log $I = -1$ and -2, bleached about 95 and 50%, respectively, of the rhodopsin content of the dark-adapted skate retina. With the lowest intensity, log $I = -5$, less than 0.1% of the rhodopsin molecules were isomerized. Data from three representative experiments. In all experiments, *b*-wave and isolated receptor potential data were obtained from same eyecups.

pigment concentration and is often termed "neural" adaptation; the latter relates to the regeneration of rhodopsin and is generally referred to as "photochemical" adaptation (Dowling, 1963; Frank and Dowling, 1968). However, the interesting feature of these data is that although both electrical potentials appear to exhibit the two components of dark adaptation, they adapt at the same rate only during the photochemical phase. Immediately after the light-adapting exposure, there is a silent period, during which time neither potential can be elicited (Dowling and Ripps, 1970). However, the receptor potential becomes responsive sooner and the neural phase of adaptation is completed earlier than for the *b*-wave.

With a 10-fold weaker adapting light (log $I = -2$) less visual pigment is bleached, the fast component constitutes a greater part of the dark-adaptation

process, and the disparity between the *b*-wave and receptor potential during neural adaptation is more pronounced. But again the two functions converge after about 10 min of dark adaptation, and thereafter (during the photochemical phase) thresholds follow the same time-course.

The lower set of data of Fig. 12 completes the picture. The dim adapting field ($\log I = -5$) bleached an insignificant fraction of the visual pigment, and consequently only the neural phase of dark adaptation is evident. The photoreceptors dark adapt almost instantaneously, but the *b*-wave results show that network adaptation still requires more than 10 min for completion. In fact, comparing the initial segment of the *b*-wave curves for the various intensities of light adaptation suggests that they are of the same form, but displaced on the scale of ordinates in accordance with the threshold-elevating effects of bleached photopigment (Dowling and Ripps, 1970). This finding is consistent with the earlier observation that the time-course of neural dark adaptation appears to be more dependent on the duration of the light exposure than on its intensity (Fig. 11 *a*).

DISCUSSION

Retinal Mechanisms in Visual Adaptation

The results of the present experiments indicate that two principal mechanisms regulate the changes in retinal sensitivity associated with visual adaptation in the skate. One of these is subserved by the photoreceptors, the other occurs in the retinal network, the origins of which have yet to be identified. By analyzing the adaptive properties of responses arising at different levels within the retina, it has been possible to define for the most part the conditions under which each mechanism predominates in the control of ganglion-cell thresholds, i.e. retinal output sensitivity. For example, the loss of sensitivity that results from light adaptation to dim backgrounds is due clearly to the influence of the network, since the receptors exhibit little or no change in sensitivity in these circumstances (Fig. 2). With brighter backgrounds, on the other hand, receptoral thresholds as well as those of more proximal elements increase as a linear function of adapting intensity, suggesting that the receptors govern the light-adapting process. Recent electrophysiological studies in both carp (Witkovsky et al., 1975) and ground squirrel (Green and Dowling, 1975) have provided evidence that with photopic backgrounds the incremental thresholds reflect the sensitivity levels established by the photoreceptors. If skate rods do in fact take over control of sensitivity as I_B is raised, the transition from the network to the receptors is remarkably smooth, for there was no apparent break in any of our incremental threshold functions.

The distinction between receptoral and network mechanisms is perhaps more apparent in the results of dark adaptometry. First of all, there are the dark-adaptation curves that follow exposure to very weak preadapting fields

(Figs. 8 and 12). Since the initial light-adapting effect on the photoreceptors is minimal, both the receptoral response and the S-potential return to dark-adapted levels almost immediately. But there is obviously a longer-lasting effect in the network as shown in the adaptation curves for the *b*-wave and ganglion-cell responses; these dark adapt together over a time-course of more than 10 min. Secondly even when the intensity of the preadapting exposure is sufficiently intense to bleach a significant fraction of the available rhodopsin, the early (i.e. neural) phase of dark adaptation observed at the retinal output is governed primarily by the network mechanism. However, it appears that irrespective of the adapting intensity, the processes involved in network adaptation exert their effects only for a limited period of time ($\sim$10–15 min) after the adapting light is extinguished. As shown in the upper sets of curves in Fig. 12, thresholds for the receptors and the *b*-wave converge during this period, and thereafter the thresholds for both follow the kinetics of rhodopsin regeneration, i.e. the photoreceptor mechanism becomes rate limiting.

Neural Adaptation

Each of the electrical potentials examined in this study exhibits the rapid, neural phase of dark adaptation, but it is apparent that during this phase of dark adaptation the rates of recovery of the distal responses (receptor- and S-potentials) differ from those of the responses arising more proximally within the retina (*b*-waves and ganglion-cell spikes). Moreover, the parameters of the preadapting exposure may have different effects on the two sets of responses. When different exposure times are employed to deliver equivalent numbers of quanta to the retina, the receptor potential data follow the same dark-adaptation curve for long and short durations (Fig. 11 *b*), whereas *b*-wave recovery is significantly delayed by a longer, albeit weaker exposure (Fig. 11 *a*). In this connection it should be noted that the enhanced light-adapting effect of a 5-min exposure time as compared with a 1-min duration, observed also in human dark adaptometry (Crawford, 1946), argues against the notion that visual sensitivity during neural adaptation is related to the concentration of one or more of the products of rhodopsin decomposition (Donner and Reuter, 1968; Rushton and Powell, 1972). The formation of photoproducts is likely to follow the Bunsen-Roscoe law ($I \cdot t$ = constant) for brief exposures, and any deviation from this relation for long exposure times may be expected to yield a lower concentration of these substances (cf. Frank and Dowling, 1968). These arguments further support the view that receptoral events do not control the neural phase of visual adaptation.

Mechanisms of Network Adaptation

It has long been maintained that signal attenuation by an automatic gain control is responsible for the fact that incremental threshold functions (Fig.

4) obey Weber's law, i.e., $\Delta I \propto I_B$, rather than the square-root relation derived from fluctuation (noise) theory (cf. Rose, 1948; Barlow, 1965). Recently, Enroth-Cugell and Shapley (1973) have suggested that this facet of adaptation is controlled by the nonlinear feedback of horizontal-cell potentials onto photoreceptors. It is presumed that the light-evoked membrane potential of the horizontal cell attenuates signals from the pool of receptors that drive the bipolar cell, and this effect in turn, depresses the sensitivity of the ganglion cell. Our findings, in which the membrane potential and photic sensitivity of the horizontal cell were monitored directly during light adaptation, are clearly at odds with this hypothesis. Indeed, increment thresholds measured proximal to the network mechanism (i.e. for the *b*-wave and ganglion cell) appear to be entirely unrelated to the response properties of the horizontal cell (Figs. 4 and 5).

The threshold changes that occur during the course of dark adaptation also run counter to the idea that the horizontal cell is the principal element of the network mechanism. Both the membrane potential and the sensitivity of the horizontal cell recover quickly to dark-adapted levels (Figs. 8 and 10; Dowling and Ripps, 1971), whereas network adaptation continues for a much longer time after the adapting light is extinguished. It should be stressed that neither Enroth-Cugell and Shapley (in cat) nor we (in skate) have determined by intracellular recording the effects of horizontal cell potentials on either receptors or bipolar cells. However, in the turtle and mudpuppy, where this interaction has been studied, the inhibitory effects of horizontal-cell polarization subsides within a fraction of a second after the light stimulus is withdrawn (Baylor et al., 1971; Werblin and Dowling, 1969; Werblin, 1974; Yazulla, personal communication).

Perhaps the most striking features of network adaptation are the unusually prolonged effects of weak illumination, none of which are manifest by the receptors (Figs. 7 and 8). In fact it is difficult to conceive of a neural "filter" or synaptic mechanism with time constants approaching those required to account for the slow changes in increment threshold after the onset of a luminous field, or the slow recovery of threshold that follows its cessation. There is the possibility that nonsynaptic events are responsible for these effects. For example, illumination could cause a desensitizing substance, e.g., a neurotransmitter or an ion, to accumulate in the distal retina where it affects selectively elements controlling retinal sensitivity. Some preliminary experiments have indicated that an excess of potassium ions in the retinal extracellular milieu reduces the sensitivity of the *b*-wave response without significantly altering receptoral thresholds. Future experiments on the ionic changes in the retina induced by illumination may provide further insights into the mechanisms of network adaptation.

We thank Patricia A. Sheppard for preparing the drawings.
This research was supported in part by grants (EY-00824, EY-00379, and EY-00285) from the National Eye Institute, U.S. Public Health Service and by an award in memory of Harry Groedel from Fight-for-Sight, Inc., New York.

Received for publication 5 July 1974.

REFERENCES

BARLOW, H. B. 1965. Optic nerve impulses and Weber's law. *Cold Spring Harbor Symp. Quant. Biol.* **30**:539–546.

BAYLOR, D. A., M. G. F. FUORTES, and P. M. O'BRYAN. 1971. Receptive fields of single cones in the retina of the turtle. *J. Physiol. (Lond.)*. **214**:265–294.

BOYNTON, R. M., and D. N. WHITTEN. 1970. Visual adaptation in monkey cones: recordings of late receptor potentials. *Science (Wash. D.C.)*. **170**:1423–1426.

BROWN, K. T. 1968. The electroretinogram: its components and their origin. *Vision Res.* **8**:633–677.

CRAWFORD, B. H. 1946. Photochemical laws and visual phenomena. *Proc. R. Soc. Lond. B Biol. Sci.* **133B**:63–75.

DONNER, K. O., and T. REUTER. 1968. Visual adaptation of the rhodopsin rods in the frog's retina. *J. Physiol. (Lond.)*. **199**:59–87.

DOWLING, J. E. 1960. The chemistry of visual adaptation in the rat. *Nature (Lond.)*. **188**:114–118.

DOWLING, J. E. 1963. Neural and photochemical mechanisms of visual adaptation in the rat. *J. Gen. Physiol.* **46**:459–474.

DOWLING, J. E., and H. RIPPS. 1970. Visual adaptation in the retina of the skate. *J. Gen. Physiol.* **56**:491–520.

DOWLING, J. E., and H. RIPPS. 1971. S-Potentials in the skate retina: Intracellular recordings during light and dark adaptation. *J. Gen. Physiol.* **58**:163–189.

DOWLING, J. E., and H. RIPPS. 1972. Adaptation in skate photoreceptors. *J. Gen. Physiol.* **60**:698–719.

EASTER, S. S. 1968. Adaptation in the goldfish retina. *J. Physiol. (Lond.)*. **195**:273–281.

ENROTH-CUGELL, C., and R. M. SHAPLEY. 1973. Adaptation and dynamics of cat retinal ganglion cells. *J. Physiol. (Lond.)*. **233**:271–309.

FABER, D. S. 1969. Analysis of the slow transretinal potentials in response to light. Ph.D. Thesis. University of New York at Buffalo.

FRANK, R. N., and J. E. DOWLING. 1968. Rhodopsin photoproducts: effects on electroretinogram sensitivity in isolated perfused rat retina. *Science (Wash. D. C.)*. **161**:487–489.

GRABOWSKI, S. R., L. H. PINTO, and W. L. PAK. 1972. Adaptation in retinal rods of axolotl; intracellular recordings. *Science (Wash. D. C.)*. **176**:1240–43.

GREEN, D. G., and J. E. DOWLING. 1975. Electrophysiological evidence for rod-like receptors in the gray squirrel, ground squirrel and prairie dog retinas. *J. Comp. Neurol.* **159**:461.

KANEKO, A. 1971. Electrical connexions between horizontal cells in the dog-fish retina. *J. Physiol. (Lond.)*. **213**:95–105.

KAPLAN, I. T., and H. RIPPS. 1960. Effect on visual threshold of light outside the test area. *J. Exp. Psychol.* **60**:284–289.

KOLB, H. 1970. Organization of the outer plexiform layer of the primate retina: electron microscopy of Golgi-impregnated cells. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **258**:261–283.

MILLER, R. F., and J. E. DOWLING. 1970. Intracellular responses of the Müller (glial) cells of the mudpuppy retina: their relation to *b*-wave of the electroretinogram. *J. Neurophysiol.* **33**:323–341.

NORMANN, R. A., and F. S. WERBLIN. 1974. Control of retinal sensitivity I. Light and dark adaptation of vertebrate rods and cones. *J. Gen. Physiol.* **63**:37–61.

ROSE, A. 1948. The sensitivity performance of the human eye on an absolute scale. *J. Opt. Soc. Am.* **38**:196–208.

RUSHTON, W. A. H. 1961. Dark adaptation and the regeneration of rhodopsin. *J. Physiol. (Lond.)*. **156**:166–178.

RUSHTON, W. A. H. 1965 *a*. Visual adaptation. *Proc. R. Soc. Lond. B Biol. Sci.* **162**:20–46.

RUSHTON, W. A. H. 1965 *b*. The sensitivity of rods under illumination. *J. Physiol. (Lond.)*. **178**:141–160.

RUSHTON, W. A. H., and D. S. POWELL. 1972. The early phase of dark adaptation. *Vision Res.* **12**:1083–1093.

WERBLIN, F. S. 1974. Control of retinal sensitivity II. Lateral interactions at the outer plexiform layer. *J. Gen. Physiol.* **63**:62–87.

WERBLIN, F. S., and D. R. COPENHAGEN. 1974. Control of retinal sensitivity III. Lateral interactions at the inner plexiform layer. *J. Gen. Physiol.* **63**:88–110.

WERBLIN, F. S., and J. E. DOWLING. 1969. Organization of the retina of the mudpuppy, *Necturus maculosus*. II. Intracellular recording. *J. Neurophysiol.* **32**:339–355.

WITKOVSKY, P., E. DUDEK, and H. RIPPS. 1975. Slow PIII component of the carp electroretinogram. *J. Gen. Physiol.* **65**:119.

WITKOVSKY, P., J. NELSON, and H. RIPPS. 1973. Action spectra and adaptation properties of carp photoreceptors. *J. Gen. Physiol.* **61**:401–423.