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Adaptation in a Vertebrate Retina: Intracellular Recording in *Necturus*

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THE VERTEBRATE visual system functions over an extremely broad range of luminances by adapting its operating characteristics to the prevailing conditions. Psychophysical measurements show that when luminance levels are low, the visual signal is broadly integrated over time and space, and the threshold for the system approaches that of an ideal detector (2) in which threshold is determined only by fluctuations in background illumination. At higher luminance levels, less integration takes place, both temporal and spatial resolution improve, and threshold rises to a higher level than that required of an ideal detector (1). Electrical recordings at the level of the ganglion cell in cat have shown that at least part of the adaptive process occurs within the retina itself. Barlow (1) and Barlow and Levick (4) have shown that ganglion cells in cat possess some of the psychophysically measured properties, behaving like ideal detectors at low luminance levels, and becoming less ideal at higher levels. Barlow, Fitzhugh, and Kuffler (3) showed that the antagonistic surround tended to limit the size of the center of the receptive field in cat ganglion cells only after background luminance was increased.

The evidence suggests that light adaptation is mediated by a change in organization within the retina. The sites of these changes and their properties remain obscure although intraretinal recordings have provided some clues as to where adaptation may take place. Brown and Watanabe (7) found that when they stimulated the retina of monkey with a fixed intensity flash, the magnitude of the b-wave of the electroretinogram was reduced by light adaptation but the magnitude of the a-wave remained constant. They suggested that there exists a neural stage of adaptation between receptors and the inner nuclear layer of the retina where the b-wave was generated. Dowling (8) determined the sensitivity of the electroretinogram in rat at different levels of light adaptation and found that the a-wave saturated at background levels that only reduced the sensitivity of the b-wave. He concluded that the site of adaptation was related to the cells that gave rise to the b-wave, tentatively identified as bipolars, but more recently Miller and Dowling (11) have shown the Müller cells may be the generators of the b-wave in the retina, and that they are affected by activity at the outer plexiform layer of the retina.

The evidence from intracellular recording is not yet sufficiently detailed to locate the adaptive mechanisms. Witkovsky (19) showed that the L-type S potentials in carp do not adapt but saturate with increasing background, but Naka and Rushton (13) found that the range of response of the L-type S potentials in tench could be shifted to higher luminance levels by bleaching visual pigment. Studies at the bipolar level suggest that some form of adaptation may take place there. Werblin and Dowling (18) showed that the sensitivity of the bipolar cell to stimulation at the center of its receptive field could be reduced by including illumination at the periphery of the field, and Werblin (17) showed that the antagonistic surround for bipolar cells exists only in the light-adapted retina.

To summarize, changes in sensitivity due to background illumination, as measured psychophysically, appear at the level of the b-wave generator and ganglion cells in the retina but not at the a-wave or in the S potential generators. Reduction of spatial summation, measured psychophysically, may be related to the appearance of an antagonistic surround in the light-adapted retina, as shown for ganglion cells in cat and in bipolar cells in mudpuppy.

The retina of the mudpuppy, *Necturus maculosus*, is a useful preparation for studying the mechanisms of adaptation because the cells are large enough to be easily penetrated by micropipettes, and the time course of response to flashes has been established for cell types identified by intracellular staining (17). Receptive fields for each type of cell have been described for both flashing (17) and moving targets (18), showing that the mudpuppy retina possesses many of the characteristics of a typical vertebrate retina, and the fine structure of this retina has been studied so that synaptic relations between cell types are known (9).

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METHODS

Stimulator

These experiments were designed to determine how the background luminance affected the response characteristics, including gain and dynamic range, of each type of retinal cell. To accomplish this a special stimulator was designed to perform two functions. It first introduced a fixed background luminance, and then generated small, stepwise incremental changes in luminance around that background level. Light originated at a tungsten source, was diffuse, and covered the range of intensities within 6 log units of ganglion cell threshold in these experiments. The system, shown in Fig. 1, used moving neutral density filters to generate a "staircase" of ½ log unit step increments in intensity covering a 4-log unit range in 5 sec.

The following procedure was used during a typical experiment. After a retinal unit had been penetrated and identified by criteria described in previous work (18), a fixed background level was introduced and maintained until no further change in intracellular potential was observed. This usually took less than a minute, after which the staircase generator was stepped down in intensity. Then incremental stepwise changes in intensity were generated starting at a level far enough below background that the entire response range for the unit could be measured. The continuous curve in Fig. 2 represents the result of such an experiment. This is a Polaroid photograph of the face of an oscilloscope where the position of the neutral-density wedges (shown in Fig. 1) was monitored and used to deflect the oscilloscope beam horizontally at 1 log unit per large division, and the response of the unit deflected the beam vertically, so the curve represents the log intensity-response relation for the horizontal cell.

The level of background luminance is indicated by the vertical arrow in Fig. 2 and subsequent figures. Notice that the arrow does not correspond to threshold intensity but falls about one-third of the way along the curve from threshold to saturation. Thus, this horizontal cell generated a graded response over intensities that include but do not begin at background.

Vertical bars representing the peak responses of the same cell to flashing stimuli at different intensities are also included in the figure. The

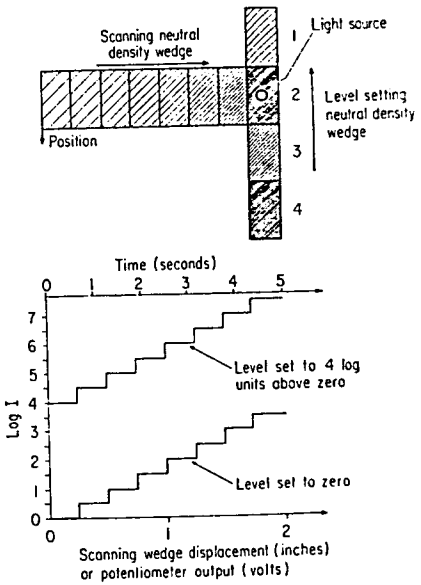

FIG. 1. Moving wedges for generating the staircase stimulus intensity-response functions. The horizontal wedge increases in eight ½ log unit steps of density, covering a total range of 4 log units in 5 sec. The absolute luminance range covered by the moving wedge is set by the vertical wedge, with variable density of 5 log units. The form of the stimulus intensity versus position and time is shown below for increasing intensities at two settings of the vertical wedge.

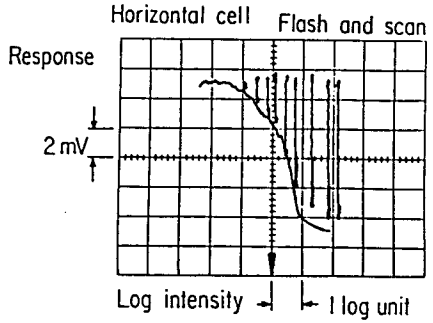

FIG. 2. Comparison of the peak response to flashing stimuli of increasing intensity (vertical lines) with the intensity-response relation generated by the staircase stimulator (continuous curve). Vertical arrow indicates background luminance. Both techniques generate comparable curves.

peak responses for flashing stimuli fall along the continuous curve, indicating that a similar curve can be generated by either flashing or stepping the stimulus.

Preparation

As described previously (18), the retina of the mudpuppy was penetrated through the vitreous, after the anterior eye including the lens had been cut away. The retina remained in the eyecup which remained in the decapitated head. No special efforts were made to oxygenate the preparation, to remove the vitreous from the retina, or to keep the preparation moist. Under these simple conditions the retina remained functional for at least 4 hr, with little measurable change in the characteristics of the response types.

Electrodes

Pyrex capillary tubing, filled with a few strands of Fiberglas, was pulled on a Livingston-type pipette puller to tips about 0.1 μ in diameter. The electrodes were filled with 1 M potassium acetate and the tip resistances were typically 350 megohms, but electrodes having twice or half this resistance were used successfully. The electrodes were coupled to the recording amplifier through a silver-silver chloride wire that was connected to the gate of a field effect transistor (FET) used as a source follower. The FET had an input resistance rated at 10^{13} ohms and an input capacitance of 2 pf. Long-term measurements were needed in this study so the "grid current" of the FET was reduced to a minimum by supplying the gate with a controllable bucking current to eliminate effects of polarization at any of the junctions in the path from cytoplasm to the gate of the FET.

For simultaneous recordings from two retinal units, a pair of electrodes was "glued" together with dental impression compound as their tips were positioned to within 100 μ under a microscope. Both electrodes were driven into the preparation together by a single syringe-type hydraulic drive.

Reading response curves

All results except Figs. 5 and 8 are presented in the same format in this report. The intensity-response curves were plotted directly on the face of an oscilloscope with response on the ordinate, calibrated at 1 or 2 mv/large division, and stimulus intensity calibrated at 1 log unit/division recorded on the abscissa. The approximate range of intensities used was between 10^1 and 10^6 quanta incident/sec per receptor, so most of the observed changes in response probably do not involve bleaching of visual pigment (8). Absolute intensities are not necessarily in register for different figures, but relative thresholds for different cell types are obtainable for figures where simultaneous recordings from two units are presented (Figs. 8 and 10). The range of intensities covered in any single recording, usually about 4 log units, can be read as the horizontal distance between the ends of each continuous curve. All stimulation was diffuse, so the term "background level" is meant to specify the steady luminance level that was maintained until the stepwise stimulus was applied. The approximate background intensities for each set of recordings are shown by the vertical arrows in the figures along the intensity axis. Intensity-response curves generated by decreasing intensities of the staircase stimulator are not shown, but they are predictable from the curves to increasing intensity included in this report.

RESULTS

Intensity-response curves for receptors at different background luminances

The intensity-response curves for receptors, shown in Fig. 3*A*, were generated by the following procedures. First one of four background levels indicated by the vertical arrows was selected and maintained until no further change was observed in receptor polarization. The initial (leftmost) portions of the four superimposed curves indicate the potential to which the receptor hyperpolarized at each background level.

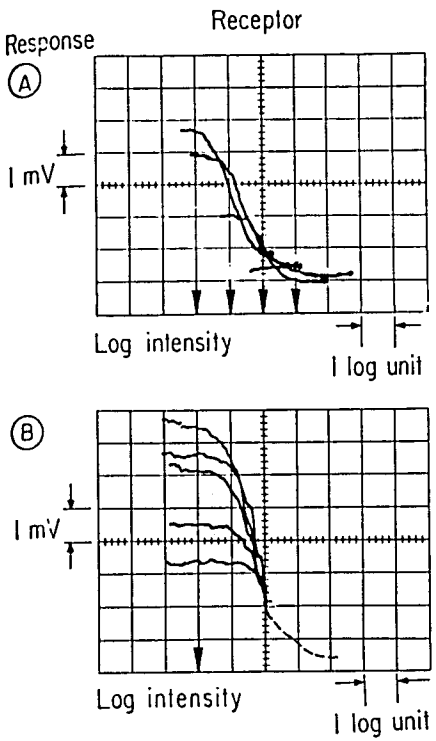

FIG. 3. Receptor response. *A:* each curve represents the total intensity-response relation generated by staircase stimuli starting near each background level (vertical arrows). Curves have been adjusted vertically to bring the saturation levels into alignment (see text), and together form a single, continuous fixed curve. *B:* curves were obtained at 0.5-min intervals after the background in *A* was reduced by 3 log units to vertical arrow. Each subsequent curve is higher than the last. Highest curve represents original resting potential. Dashed curve represents approximate high-intensity range of curve.

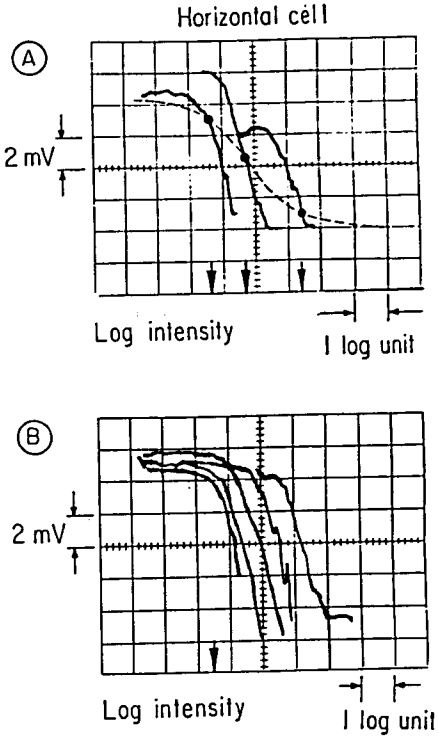

FIG. 4. Horizontal cell response. *A:* the three curves represent the response of the horizontal cell to the staircase of intensities at three different background levels (vertical arrows). The background levels generated a hyperpolarizing potential as shown by the large dots connected by the broad, dotted curve. *B:* each curve represents the intensity response relation in the horizontal cell at 0.5-min intervals after the background level was reduced by 3 log units to the vertical arrow. Each subsequent curve falls further to the left. Leftmost curve represents the dark-adapted intensity-response relation.

Then, the staircase stimulator was run back so that the measurement started at an intensity below background and increased in ½ log unit steps as described in METHODS. As the stimulus intensity increased above each background level the receptor hyperpolarized with each step of the stimulator until it saturated.

The initial portions of the curves in Fig. 3*A* are horizontal because receptors do not appear to respond rapidly to increments in intensity from zero up to a level near background. When background level was reduced, the receptor potential returned to the original dark level over a long time course, measured by the experiment shown in Fig. 3*B*. Here, the background level was reduced by 3 log units to the level indicated by the vertical arrow and incremental stimuli falling mostly below the response range for the receptor were used to test the response at 0.5-min intervals. The base line

moved upward, so the first curve in the series is the lowest; the last is the highest. These data were used to plot the return of potential as a function of time in Fig. 7. Since relatively low intensities were used in the experiment shown in Fig. 3*B*, the entire intensity-response curve, including the saturation levels, was not generated, so the dotted line has been added to the curves to show, approximately, how they would be completed.

Receptor responses were quite small, usually only about 1–2 mv in magnitude, so the long term d-c measurements required in these experiments were often subject to distortion by drift in potential level. The effects of drift have been compensated for in Fig. 3*A* by aligning the curves vertically so that all the saturation levels coincide. This seemed to be a reasonable procedure because pairs of curves generated at different background levels usually had coincident saturation levels. When vertical adjustment was made in this and similar experiments, the four response curves taken at different background levels fell along a single, more extended common curve. If alignment of the saturation levels is justified then the apparent effect of background illumination in receptors is simply to shift the starting point for the graded response to illumination along a single curve. The position of the total, common curve seems to be unaffected by background level.

These responses are probably recorded from cones because the peak of the action spectra lies near 575 nm, which is the peak of P. A. Liebman's (personal communication) microspectrophotometric absorption curves for mudpuppy cones but not for rods, whose peak absorption is at 525 nm. Also, Toyoda et al. (16) recorded only from cones in mudpuppy under similar experimental conditions. The dynamic range for receptors in this study appears to be wider than that determined by Tomita et al. (15) in fish cones, by Baylor and Fuortes (6) in turtle cones, but not for Steinberg's (14) measure of the cone response component in cat horizontal cells. The difference may be due to the experimental techniques used here, in which maintained background levels were presented to the retina before the response function was measured.

Intensity-response curves for horizontal cells at different background luminances

Procedures for generating the horizontal cell intensity-response curves shown in Fig. 4*A* and *B* were identical to those used above for receptors. After presenting each background level (vertical arrows) the light intensity was first reduced and then incremented in ½ log unit steps so that the intensities of the staircase stimulator covered the full range of graded hyperpolarization in the horizontal cell from threshold to saturation.

In horizontal cells the form of the response curve was different for rapidly and slowly changing stimuli. Figure 4*A* shows that increasing fixed background levels (vertical arrows) elicited increasingly greater hyperpolarizations in the horizontal cell. These points have been connected by the dashed curve in the figure. At each increased background level the response to the more rapid incremental stimuli generated a whole new curve, roughly parallel to its neighbor but shifted to the right along the log-intensity axis.

The threshold level for each curve fell below the corresponding background level (vertical arrows), indicating that background fell at an intermediate point along the graded response curve. There was, in fact, a trend as background level was increased for more of the graded response range to fall below background level as shown in Fig. 4*A*. To clarify this phenomenon, Fig. 5 shows the time course of response for a horizontal cell to flashing stimuli at three background levels. As background was increased, the relative magnitude of hyperpolarization at "on" decreased, while the relative magnitude of response at "off" increased. The curves in Figs. 4*A* and 5 both show that, for stimuli at any background, the membrane potential is confined between two fixed absolute boundaries corresponding to threshold and saturation, so that at higher backgrounds where the maintained potential is held closer to saturation, most of the response range lies at lower intensities.

When the background level was reduced, the intensity-response curves shifted slowly in time to the left back to the original dark-

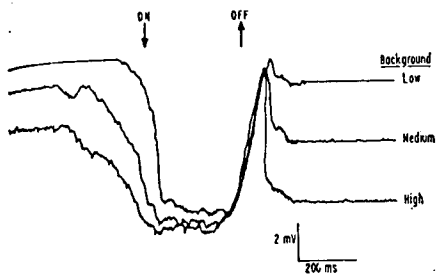

FIG. 5. Time course of horizontal cell response to flashes at increasing background intensities. Backgrounds increase in log unit increments and diffuse flashes were 1 log unit above each background. As background was increased, the horizontal cell became hyperpolarized and more of the response was generated in the off direction back toward the original base line. Onset and cessation of flash shown approximately by arrows.

background level, remaining roughly parallel. A series of curves was generated to measure the time course of the effect, shown in Fig. 4*B*. Here the background level was reduced abruptly by 3 log units to the level indicated by the arrow, and an incremental series of intensities was generated every 0.5 min through levels mostly below the response range for the horizontal cell. Each subsequent curve is shifted to the left, and the shift of the points of inflection in these curves is plotted against time in Fig. 7.

Reference to Fig. 4*A* shows that as background intensities were increased, each new response curve fell at a range of intensities in the saturated region of its left neighbor. The mode of transition from saturation to responsiveness with respect to an elevated range of intensities is illustrated in Fig. 6. Here, the background level was suddenly increased by 3 log units and instantaneous intensity-response curves were plotted by the methods described above. The intensity-response curve generated before the background increase is marked as a. At 10 sec after the shift a nearly saturated response (b) was elicited, but this response continued to grow, as the plots (c and d) taken at subsequent 10-sec intervals reveal. The dotted, vertical lines in the figure are drawn through the points of inflection in the curves to help illustrate that the points of inflection for the curves b, c, and d are nearly coincident and located about 2 log

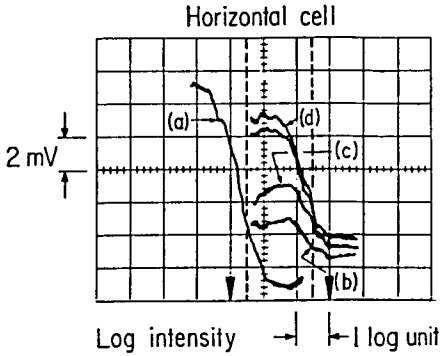

FIG. 6. Two phases of adaptation in the horizontal cell. Curve a is the intensity-response relation before luminance level was increased by 3 log units (vertical arrows). Curves b, c, and d were generated at 10, 20, and 30 sec after the increase. The point of inflection shifts almost immediately (b), but the resting potential rises back to the base line more slowly (c, d).

units above the point of inflection for the original curve, a.

The recordings from horizontal cells were quite stable, and in these experiments responses at all adaptation levels could be repeated many times with the same unit. Therefore, no realignments were made in presenting the curves shown in Fig. 4. Recordings for 11 units gave consistent results over the entire intensity range studied here. Other units were lost before a complete study was performed, but these results were also consistent, except that there was variation in the absolute magnitude of response and absolute threshold from cell to cell.

Many units in the distal retina had properties intermediate between receptors and horizontal cells (showing both a downward and rightward shift in the curves with increasing background illumination). These units had the broad receptive fields and long latencies associated with horizontal cells (18) and must represent either another type of horizontal cell or one more closely coupled, somehow, to the receptor activity.

Response of bipolar cells at different background luminances

Previous studies of the bipolar cell in *Necturus* (17, 18) have indicated that the light-evoked response results from the inter-

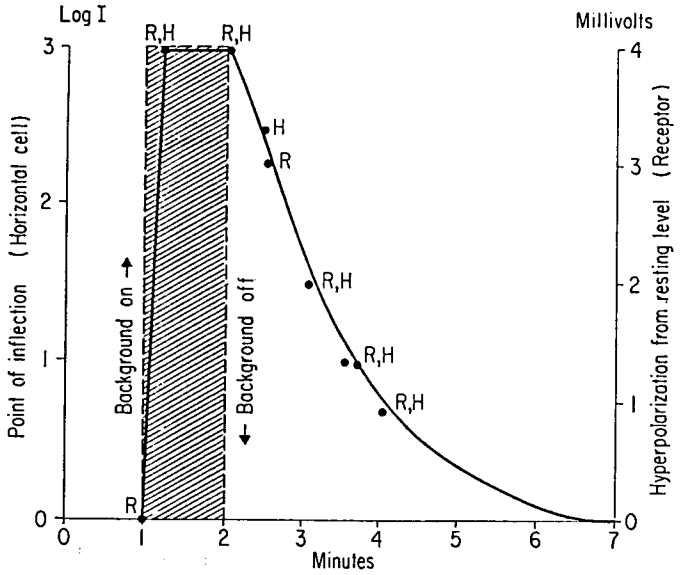

FIG. 7. Time course of potential change in receptor (R) and shift of inflection point in horizontal cell (H). Curves show the rapid shift in resting potential and inflection point with increasing background, contrasted with a relatively slow decay after decreasing background luminance.

action of antagonistic components elicited at the center and surround in the receptive field. These separate components can be identified even when diffuse stimulation is used because the central effect precedes the peripheral effect by about 200 msec. For example, Fig. 8*A* shows that the response of a bipolar cell illuminated centrally is a sustained hyperpolarization, but Fig. 8*B* shows that when both center and surround are illuminated in the same cell, the center response occurs first and after 200 msec the antagonistic surround "turns it off." These results can be used to interpret the curves generated in response to the stepwise stimulator used in the present experiments.

Figure 9 shows the intensity-response curves recorded simultaneously from a horizontal cell and a bipolar cell. The bipolar cell had a lower threshold and required a narrower range of intensities to pass from threshold to peak than the horizontal cells. But, whereas the peak response in the horizontal cell represented a saturation, the peak in the bipolar response was reduced after 200 msec by the antagonistic surround, and interactions between antagonistic components extended over a broad range of intensities, as illustrated by the fluctuations in potential in the figure.

The initial portion of the intensity-response curve for bipolar cells could be shifted along the intensity axis to the right much like the horizontal cell curves described above. Figure 10 shows this type of shift for both a hyperpolarizing and a depolarizing unit. As the background intensities were increased, more of the response range fell below the background level. The effect of surround on the central response tended to increase at higher background levels as shown in Fig. 10*A*, but varied in its total effect from unit to unit, whether of the depolarizing or hyperpolarizing variety. In Fig. 10, for example, the surround effect is strong in the hyperpolarizing unit with a fluctuation at each ½ log unit step, but this effect is not noticeable in the depolarizing unit.

More than 40 bipolar cells were studied, all generating quite stable responses so no adjustment of the levels for the curves was made at different luminances. It is striking, therefore, that little relation seems to exist between steady luminance level and resting potential in bipolars. Simultaneous recordings from pairs of bipolars indicated that thresholds, points of inflection, and saturation levels occur at similar intensities for the entire population at a given adaptation level.

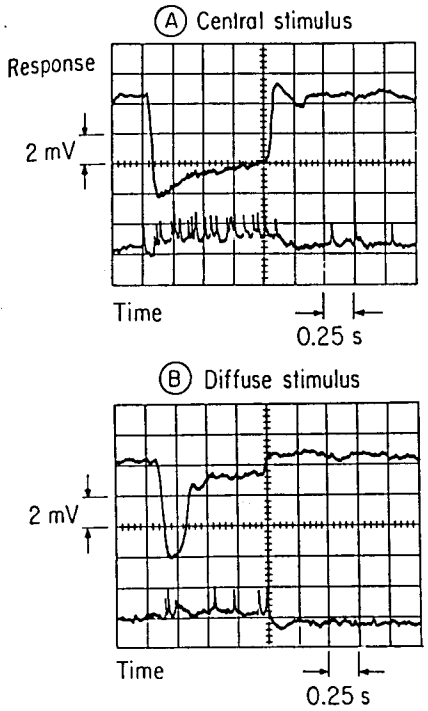

FIG. 8. Bipolar response to flashing stimuli. Central flash elicits sustained response in *A*, diffuse flash elicits a transient response in *B*, because surround turns off the center after about 200 msec. Lower traces in both figures show a ganglion cell with similar response properties recorded simultaneously with this bipolar.

Responses of amacrine and ganglion cells at different background levels

The amacrine and ganglion responses were graded over an extremely narrow range of intensities, so their activity was not easily studied under the present experimental conditions. However, a study of recordings from these units under the same stimulus conditions as used for the more distal cells is necessary to determine the relative sensitivities of neurons throughout the retina, and to follow adaptive changes generated in the distal elements through to the retinal output.

Figure 11 shows that the range of amacrine cell response, recorded simultaneously with a horizontal cell, shifted to the right as background level was increased, and that the fluctuations in the response, as the stimulator stepped through an intensity series, became better defined at the higher luminance levels. These properties of the amacrine cell response reflect the activity of bipolar cells by which they are driven; the threshold for bipolars shifts along the intensity axis, and surround antagonism becomes more effective as background levels are increased.

Figure 12 shows the responses for an on and an off ganglion cell to increasing incremental stimuli at two background levels. The threshold for sustained firing (or inhibition) in the on- and the off-unit shifted along the intensity axis by an amount comparable to that for more distal units as background was increased. The range of intensities for which the membrane potential is graded appears to be even narrower than that for bipolars, covering less than 1 log unit, and these units generate a sustained response for all intensities above the adapted threshold value. When recorded simultaneously with bipolar cells, the on-off ganglion cells generated action potentials whenever the bipolar membrane potential was changing rapidly. The correlated responses for bipolar and ganglion cells are not shown because they were more easily heard than seen.

DISCUSSION

Measurement with staircase stimulator

These experiments were designed to measure the effects of background on the characteristics of response for retinal cells after the background condition was terminated but before conditions due to a specific background had changed appreciably. The measurements made with the staircase generator shown in Fig. 1 were complete within about 5 sec and the question is whether this is fast enough. The

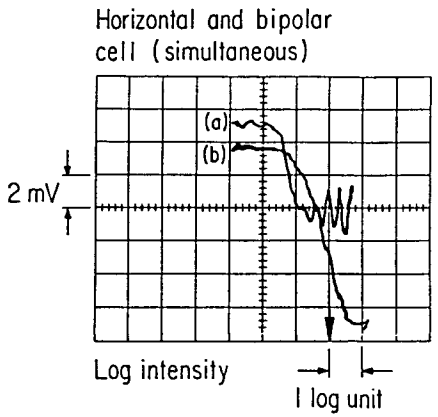

FIG. 9. Simultaneous recording from horizontal (b) and bipolar cell (a). curves show that the bipolar cell threshold is slightly lower, and the range of response narrower for the bipolar than that for the horizontal cell. Transient bipolar responses to each increment continue for the range of graded response in the horizontal cell.

answer is found in Figs. 3, 4, 6, and 7, which indicate that changes in response characteristics are complete after 30 sec following an increase in background and after 2–3 min following a decrease in background. Adjustments in response characteristics, therefore, seem to occur sluggishly enough that they are essentially "frozen" during the 5 sec required for their measurement.

Comparison of response characteristics for different cell types

The results of this study indicate that the level of steady background illumination affects the intensity-response characteristics for each type of retinal neuron. Figure 13, abstracted from the data presented above, summarizes these results by showing the intensity-response relations for each type of cell at two different background levels (vertical arrows in the figure). The stippled areas show that the response for each cell type was graded over a narrower range of intensities proceeding proximally (downward) through the retina. This trend appeared at all levels of background luminance. The intensity for apparent threshold response also became lower, but by less than a log unit proceeding proximally from receptors to ganglion cells.

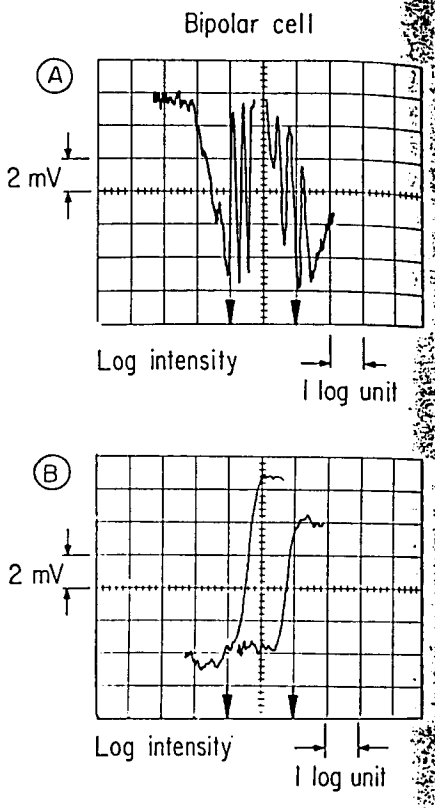

FIG. 10. Bipolar response. Each curve represents the response of the bipolar cell to ½ log unit steps of increasing intensity for two different background levels. Curves for hyperpolarizing (*A*) and depolarizing (*B*) bipolars have similar initial slopes going from threshold to saturation in about 1 log unit, and curves each shifted by about the same amount when luminance levels were increased. Fluctuations in hyperpolarizing response at each ½ log unit represent antagonistic interactions of step response explained more fully in the text.

As the background level was increased, the intensity-response curves generated by the staircase stimulator tended to shift to the right along the abscissa, but in different ways depending on cell type. In receptors, increased background luminance shifted the starting point for the graded response downward and to the right along the single curve shown in Figs. 3*A* and 13, so these receptors aproached saturation. In horizontal cells, however, increased steady background luminance caused the intensity-

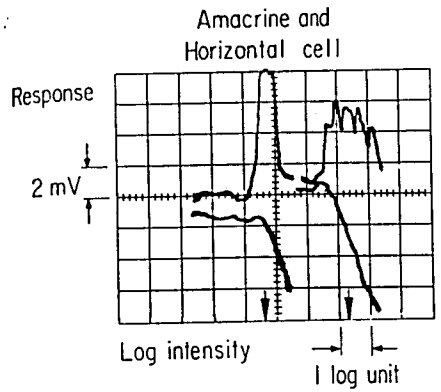

FIG. 11. Simultaneous recording from horizontal cell and amacrine cell. The threshold and range of response for the amacrine cell (upper) shift along with the horizontal cell, remaining slightly lower at both luminance levels (vertical arrows). The amacrine cell, like bipolars, is more responsive to the steps in intensity at the higher background level.

response curve elicited by the staircase stimulus to shift to the right, roughly parallel to its original form as shown in Figs. 4*A* and 13 so these horizontal cells were not easily saturated by background. The steady background levels also generated a graded hyperpolarization, shown by the dotted curves in Figs. 4*A* and 13, which extended over a broader range of intensities than any single curve generated by the staircase stimulator. These results confirm the conclusions of earlier work; that receptors saturate (8) but that more proximal units "adapt" (7, 8) as background level is increased.

As background levels were increased, the graded receptor response to rapid incremental stimuli shifted downward along a single curve to accomodate a higher range of intensities. Under similar conditions the horizontal cell response to rapid incremental stimuli shifted to the right along a curve parallel to the original. The transition from downward shifting to rightward shifting between receptors and horizontal cells is shown "in slow motion" in Fig. 6. Here, the intensity response curves, labeled b, c, and d, show that within the 30 sec following a background increase the horizontal cell response changed from nearly

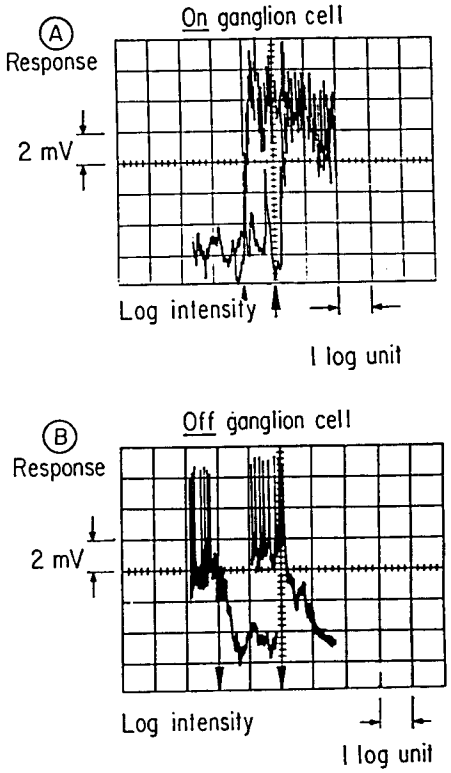

FIG. 12. Ganglion cell responses. Shifts in threshold and range of response for the ganglion cell due to increased background luminance. *A:* on-type of ganglion cell. *B:* off-type ganglion cell. The graded range of response in these cells covers only a fraction of a log unit.

saturated to fully shifted to the right. This appears to be a two-stage process. First, the point of inflection in the curve shifted to its final value within the 5 sec required to make the measurement. Second, the magnitude of the response increased as the base line shifted until it was approximately as large as the original response.

When background luminance was reduced abruptly, the response curves shifted slowly back to their original forms in characteristically different ways. In receptors, the starting point for the response shifted to lower intensities along the response curve, so the base line for the response appeared to move upward, as measured in Fig. 3*B*. In horizontal cells, the incremental response

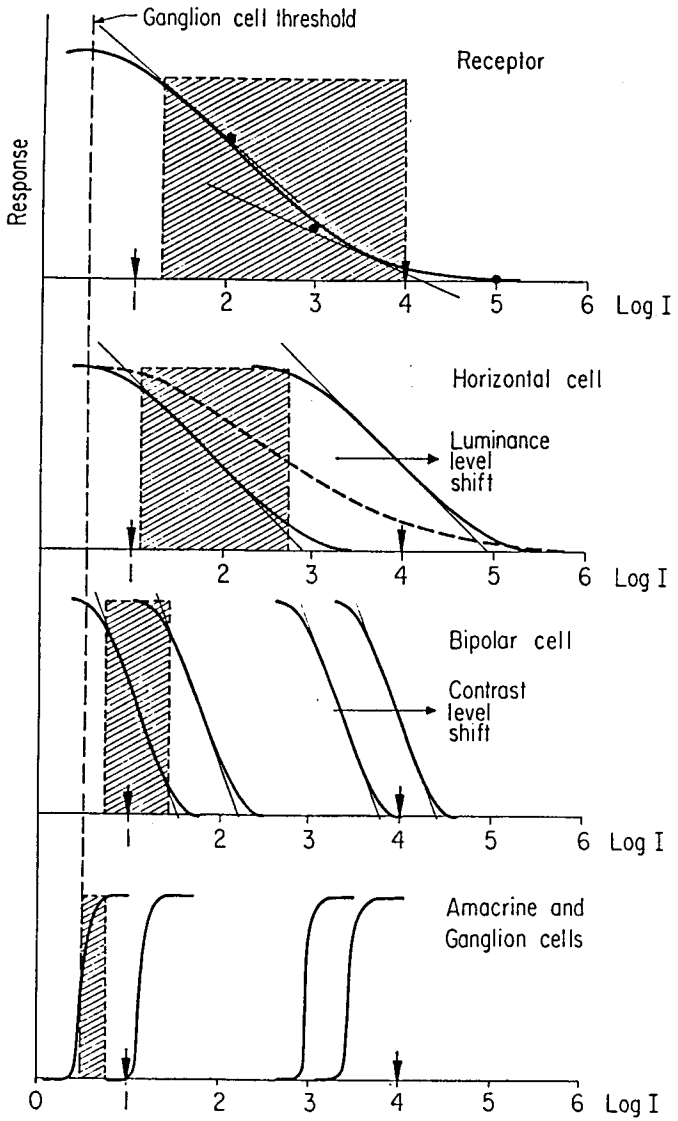

FIG. 13. Comparison of gains and dynamic ranges of response. The range of response (width of stipple) becomes narrower proceeding proximally through the retina, but the narrower curves are shiftable within the response range of the preceding neurons. Horizontal cell responses are shifted by varying background luminance levels within the range of the receptor response. Bipolar cell responses can be shifted by background or by varying surround intensity in their antagonistically organized receptive fields. The broad dotted curve shows d-c level in horizontal cell as a function of background. Solid curves show response functions to staircase stimulus. Vertical arrows on abscissa show approximate background levels for all units. The vertical dashed curve shows approximate ganglion cell threshold.

curves appeared to shift to the left, as measured in Fig. 4*B*. The time courses for these shifts were similar, occurring roughly exponentially over a period of 3–4 min, as shown for receptors and horizontal cells plotted together in Fig. 7.

Bipolar cells behaved much like horizontal cells in that the response curves were shifted to the right, parallel to each other, when background luminance was increased. A previous study (18) has shown that the response range for bipolars can be shifted by changing contrast across antagonistic zones of its concentric receptive field, so pairs of curves are included at each background level for bipolar cells in Fig. 13 to indicate that both background and contrast can control the position of the response curves. Only the initial portion of the response curves is given in Fig. 13, but the total response to transients spanned a much broader range of intensities, as shown by the transient hyperpolarizations occurring at each step of the stimulator generated by the bipolar cell in Figs. 9 and 10*A*.

Amacrine and ganglion cells behaved much like bipolars in that their response curves were shifted to the right by increasing background illumination, although the range of intensities over which the response was graded tended to become narrower in the more proximal units. These units also showed an antagonistic, concentrically organized receptive field in previous studies (17, 18). The response range can probably also be shifted by added intensity in the antagonistic surround in amacrine and ganglion cells since they are driven by bipolars, but the direct experiment has not yet been performed.

Measures of adaptation in retinal units

These studies indicate that visual adaptation—the adjustment of the incremental response characteristics to the prevailing background conditions—occurs as a series of events at different retinal levels. The final result of all adaptation processes in the retina is expressed in the response characteristics at the ganglion cell level which have been extensively studied previously, by inference from psychophysics, and electrophysiologically in the cat (1–5). A general result of the ganglion cell and psychophysical studies has been that at low background levels, increment threshold increases roughly as the square root of background luminance, but at higher background levels, increment threshold tends to increase more rapidly, i.e., retinal gain is reduced roughly in direct proportion to background level. More recent results (5) have shown how differential sensitivity across antagonistic zones of the ganglion cell receptive field allows high sensitivity to be maintained in the presence of background without increase in maintained rate of firing. The present results may be used to indicate how distal cells in the retina control ganglion cell activity to fulfill these conditions.

Threshold measured in ganglion cells or psychophysically is relatively easy to determine, (3, 5) because spike activity is either present or it is not, but firing rates, graded over a very narrow range of stimulus intensities, are more difficult to analyze. However, the more distal retina is populated by units that generate slow potentials, graded over a much broader range of intensities (6, 10, 12, 13, 16), so threshold is difficult to measure but graded responses to increasing intensities are directly observable. The relative magnitude of increment threshold can be inferred from the slope of the intensity-response curve at each background luminance, so the present results can be related to increment-threshold studies by measuring the slope of the intensity-response curve at each background.

Adaptation at low background luminance

Figure 13 shows that at low background levels the ganglion cell threshold falls below the measureable response range for the more distal cells, as indicated by the dashed line at the left of the figure. Since a ganglion cell response is elicited at these stimulus intensities, receptors must be coupled to ganglion cells through the retina. But since there is little sign of recordable neuronal interaction within the retina near threshold at low background levels, it is reasonable to suppose that receptors are coupled directly to ganglion cell. Receptors can probably perform at best as quantum detectors, where threshold could be raised simply by the square root of the mean number of arriving quanta, and

their behavior may be simply reflected in ganglion cell activity.

Adaptation at higher background luminance—relation to Weber's law

At higher background levels the relatively narrow incremental intensity-response curves for bipolar cells shift to span a range of intensities determined by background level. The resting potential in most bipolars does not increase significantly with background Figs. 9 and 10*A* because the bipolar response is controlled by antagonistic center-surround components that interact to reduce the steady-state response to diffuse illumination after about 200 msec. In this way the entire dynamic range for the bipolar cell is positioned to signal small changes in luminance near background levels.

The slope of the intensity-response curves near background can be measured to determine how the sensitivity of the system changes with various background levels. If these bipolar cell response curves shifted parallel to each other, along the log-intensity axis by an amount proportional to background intensity, their slopes with respect to a linear intensity axis would decrease by 1/I, approximating Weber's law.[1] In fact, the curves shift by somewhat less than the change in background indicating that the "gain" of the system may be reduced by slightly less than that required by Weber's law.

To summarize, at low background levels there is little recordable evidence for interactions in the retina that might modify receptor-to-ganglion cell transmission, so it is reasonable that ganglion cell threshold is simply a reflection of receptor threshold characteristics, increasing roughly with square root of background luminance. At higher background levels, interactions between receptors and horizontal cells generate a bipolar cell response with resting potential independent of background and with a slope (response versus intensity) which decreases roughly in proportion to background. Bipolar cells described above possess many of the ganglion cell characteristics described previously; maintained activity does not increase (4) as background luminance increases, center-surround organization appears (3, 17, 18), and gain is reduced (5), so it would seem that most of the processes of adaptation are mediated at the outer plexiform layer of the retina through interactions between receptors and horizontal cell processes.

[1] Weber's law states that at threshold $\Delta I/I$ is constant for all I, where ΔI is the increment in intensity required for threshold and I is the background luminance. If it is assumed that the response ΔR at threshold is constant for all I, then $\Delta I/I = \Delta R$, and rearranging, the slope, at threshold, $\Delta R/\Delta I = 1/I$.

SUMMARY

Each type of neuron in the retina of the mudpuppy responded in a characteristically different way to stepwise increases of intensity. Moreover, the response of each type of neuron was altered in a characteristically different way when background was changed. The activity of each cell type was studied to determine how the changes in response resulting from the changes in background luminance level were involved in the mechanism of retinal adaptation.

Receptors responded with a polarization which was graded with intensity over a curve that spanned about 4 log units, and the relation between the response and log intensity was not affected by background except that threshold was raised. Horizontal cells responded over a narrower range of log intensity covering about 2.5 log units, but this range of response could be moved to higher intensities by increasing background illumination. The shift in response range along the log-intensity axis, representing a decrease in gain, takes place more slowly than the incremental response of the horizontal cell. Thus, adaptation in horizontal cells appears to be characterized by a decrease in gain at higher background luminances. The bipolar cells respond over a range of intensities that is even narrower than that for the receptors and horizontal cells, covering only about 1 log unit of intensity. The range of the intensity-response curve for bipolars can be shifted to the right along the log-intensity axis either by increasing uniform background luminance or by increasing stimulation at the surround of the receptive field. Thus bipolar cells appear to exhibit a change in gain to both background and contrast. The adaptational properties of amacrine and ganglion cells resemble those of the bipolars, except that the proximal units have a slightly lower threshold and respond over a narrower range of intensities covering less than 1 log unit.

Most of the adaptive processes appear to be completed at the bipolar cell level. Here, gain is reduced, the antagonistic surround appears, and the resting potential is not altered appreciably by increasing levels of background illumination. It seems that adaptation in bipolar cells always positions the narrow range of the graded response at intensities near background so that the entire dynamic range of the bipolar cells can be used to signal small changes in luminance near the background level. These properties in bipolars are carried through to amacrine and ganglion cells as well.

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