The Proximal Negative Response and Visual Adaptation in the Skate Retina

JOHN E. DOWLING and HARRIS RIPPS

From the Marine Biological Laboratory, Woods Hole, Massachusetts 02543, the Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138, and the Departments of Ophthalmology and Physiology, New York University School of Medicine, New York 10016

ABSTRACT The proximal negative response (PNR), a complex extracellular potential derived mainly from amacrine cell activity, was studied in the all-rod retina of the skate. Tetrodotoxin (10^{-6} mg/ml) did not affect either the waveform or the latency of the response, indicating that the PNR reflects the graded, nonregenerative components of the amacrine cell potential. As regards its adaptive properties, the PNR exhibited both the extreme sensitivity to weak background light and the slow time course of light and dark adaptation that are characteristic of other responses from the proximal retina. Thus, the PNR, like the *b*-wave and ganglion cell discharge, appears to reflect adaptive processes located within the neural network of the inner retina.

INTRODUCTION

In previous studies we described the adaptive properties of retinal potentials arising from different levels within the all-rod retina of the skate (Dowling and Ripps, 1970, 1971, 1972; Green et al., 1975). Our results suggest that there are two principal stages at which visual thresholds are regulated during light and dark adaptation. One adaptive mechanism is located within the photoreceptors and affects equally the sensitivities of the receptors and the horizontal cells, the other (the "network" mechanism) is located more proximally and exerts its effect on the thresholds for both the *b*-wave and ganglion cell responses. However, neither the cellular nor the ionic basis of network adaptation has been identified as yet (but cf. Dowling and Ripps, 1976).

In an attempt to further this analysis, we have examined the adaptive characteristics of the proximal negative response (PNR) of the skate retina. Several lines of evidence suggest that this complex retinal potential is mainly the extracellular expression of amacrine cell activity (Burkhardt, 1970; Proenza and Burkhardt, 1973). Because the amacrine cells are in synaptic contact with bipolar and ganglion cells, as well as with other amacrine cells (Dowling and Boycott, 1966), it is important to establish the role of these interneurons in visual adaptation. We find that the behavior of the PNR during light and dark adaptation resembles in many respects that of the *b*-wave and ganglion cell discharge. And although there are some small differences among the adaptive properties of these different responses, we conclude that PNR thresholds, like those of other responses of the proximal retina, are affected by the processes governing network adaptation.

METHODS

Small pieces of eyecup (≈0.5 mm²) were excised under dim red light from the tapetal region of dark-adapted skates (*Raja erinacea* or *R. oscellata*), drained of vitreous humor with absorbent paper, and placed in a lucite chamber under a stream of moist oxygen. The chamber, which was mounted within a light-tight Faraday cage, had embedded in its base a chlorided silver disk that served as the reference lead for the electrical recordings.

Beveled glass micropipettes (Proenza and Morton, 1975), filled with an elasmobranch Ringer solution (Cavanaugh, 1975), were used to record the PNR. The pipettes were drawn to a tip diameter of about 1.5 μm, and beveled on fine lapping paper at an angle of approximately 30° to give electrode resistances of 3–7 MΩ. The pipettes were carried by a hydraulic microdrive (David Kopf Instruments, Tujunga, Calif.), and advanced through the retina at an angle of about 60° with respect to the normal of the surface. The signals were led to a Ag/AgCl wire connected to the input stage of high impedance DC amplifier (Metametrics, Inc., Carlisle, Mass.), the output of which was displayed on an oscilloscope and recorded on a Brush penwriter (Gould Inc., Cleveland, Ohio). The transretinal electroretinogram (ERG) was monitored with a chlorided silver electrode placed on the surface of the retina; the potentials were amplified, and recorded on a second channel of the penwriter.

Photic stimuli were delivered by a dual-beam optical system that provided independent control of the intensity, duration, size, and spectral composition of the test and adapting fields (Dowling and Ripps, 1972). Both fields were of "white" light, derived from tungsten-halogen lamps, and filtered by BG 38 and KG 1 heat filters that strongly transmit wavelengths below 560 nm and absorb wavelengths of >700 nm; the retinal irradiance (unattenuated) of each, measured in the plane of the retina with a calibrated thermopile, was 1.7 mW/cm². Throughout this paper, log I values give the densities of the neutral filters used to attenuate either the test (I_t) or the background (I_B) field. Thus log $I_B = -1$ corresponds to a background illumination of 0.17 mW/cm². Focused spots of light 100–1,000 μm in diameter were used to elicit the PNR; for ERG recordings, the stimulus flash illuminated the entire piece of eyecup.

Recording the PNR

Electrode depth, spot size, and the locus of stimulation are critical factors in obtaining large, well isolated PNR potentials (Burkhardt, 1970). The procedure we found most expeditious was to lower the micropipette to the retinal surface, to center a 250-μm spot with respect to the electrode tip, and then to monitor both the PNR and the electroretinogram to a suprathreshold spot and a full-field stimulus in turn as the pipette was advanced through the retina in 5–10-μm steps. A very small PNR and a large *b*-wave were routinely recorded with the electrode at the surface of the retina, but as the pipette penetrated the retina, the former increased while the latter decreased in amplitude. Maximal PNR voltages were recorded at a depth of 70–100 μm from the vitreal surface of the retina. This depth was approximately 20–30 μm before the *b*-wave reversed polarity and horizontal cells were encountered, and corresponded roughly to the depth at which the perikarya of amacrine cells are found. After the optimal pipette position was ascertained, the stimulus spot was adjusted in orthogonal planes to give a PNR with a sharp leading edge and large amplitude. As regards the size of the stimulus stop, a diameter of 250 μm was used for most experiments; stimulation with smaller or larger spots usually resulted in higher PNR thresholds. Thus, optimal recording of the PNR required the use of small spots of light located precisely within the retinal region sampled by the microelectrode.

In all experiments, threshold refers to a just-detectable response. At threshold, the responses usually exhibited a small rise (~10 μV) in the base line level with some transient

oscillatory potentials (cf. responses to log $I_t = -8.0$ in Figs. 1 and 3). Thresholds could easily be estimated to within 0.1 log unit.

RESULTS

The PNR of the Dark-Adapted Retina

Fig. 1 shows a series of typical responses evoked by 1-s flashes of increasing intensity. To all but the dimmest flash, the PNR consists of an initial "on"

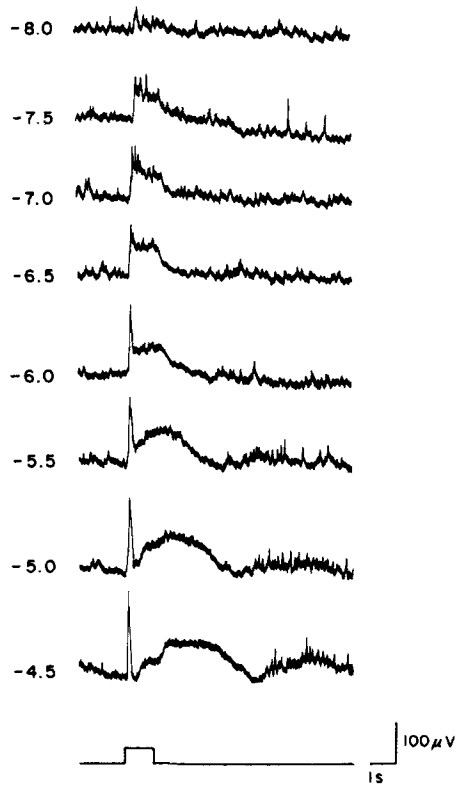

FIGURE 1. The PNR of the skate retina elicited by 1-s test flashes of increasing intensity. Note that the response to dim and moderately bright flashes (log $I_t = -7.5$ to -6.0) consists of a sharply peaked "on" transient that is followed by a plateau of lower voltage. At higher intensities (log $I_t \geq -5.5$), the plateau is converted to a broad negative wave due to the intrusion of the *b*-wave and a prolonged afterpotential. See text for details. In this and in subsequent figures, an upward deflection of the trace indicates negativity of the active electrode.

transient followed by a sustained component of lower amplitude. As the stimulus intensity is raised, the initial transient becomes more prominent and the sustained negativity increases in duration. It appears also that increasing the flash intensity alters markedly the waveform of the PNR. Note, for example, that with the brighter stimuli (log $I_t = -5.0$ and -4.5) the fast-rising on transient is followed by an equally rapid falling phase, so that the sustained component

arises from the base line of the recording. However, much of this change in waveform is probably attributable to an interaction between the PNR and the *b*-wave of the ERG. With the electrode optimally placed for recording the PNR, the polarity of the *b*-wave is opposite that of the PNR, and in response to bright stimuli the latency of the *b*-wave is sufficiently short and its amplitude is sufficiently large to affect the shape of the PNR. With relatively weak stimuli (i.e. near threshold) there was no evidence that the *b*-wave contributed to the light-evoked potential.

Fig. 2, in which the first pair of recordings was taken from Fig. 1, illustrates how at higher intensities the *b*-wave (arrows) encroaches upon the PNR. The interaction can be heightened by withdrawing the pipette slightly further from the depth at which the *b*-wave reverses polarity, or by enlarging the stimulus

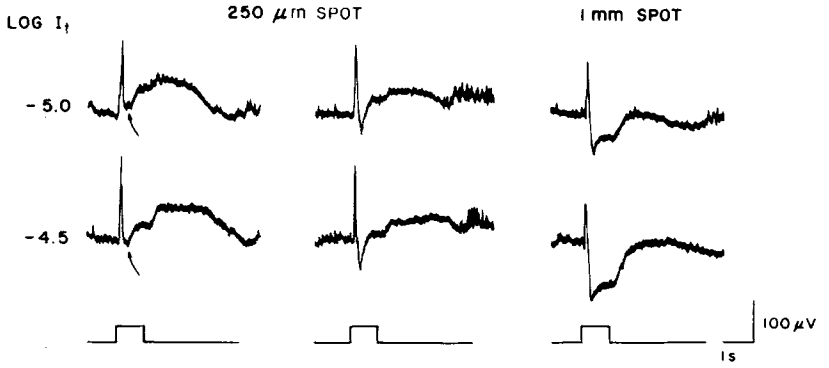

FIGURE 2. The effect of test spot diameter and electrode depth on the PNR. The two traces at the left were recorded under optimal conditions, although in both cases the bright flashes induced *b*-wave responses (arrows) that affected the waveform of the PNR. Withdrawing the electrode slightly caused the amplitude of the *b*-wave to increase (central tracings), whereas enlarging the diameter of the stimulus spot produced the greatest distortion of the PNR (traces at the right) due to a further increase in the large *b*-wave potential.

spot; either maneuver increases the amplitude of the *b*-wave relative to that of the PNR. The fact that stimulus diameter is effective in this regard is due not only to the enhancement of the *b*-wave, but also to a reduction in the PNR voltage; i.e. a consequence of the direct effect of stimulus diameter on the PNR (Burkhardt, 1969).

Some additional features of the PNR of the dark-adapted retina are shown in Fig. 3. The upper set of tracings, recorded at a fast sweep speed, suggests that the various components of the PNR consist of the sum of smaller potentials. Near threshold (log $I_t = -8.0$), for example, the fine structure of the response can be resolved into several slow transients of different amplitudes and latencies. In response to brighter stimuli, on the other hand, the on transient dominates the response and appears to be derived from a number of smaller potentials whose latencies shorten as the flash intensity is increased.

The responses shown in Fig. 3*a* were obtained from a relatively "quiet"

preparation. However, a glance at Fig. 1 reveals some of the poststimulus activity that often appeared after the sustained component had returned to the base line. In a few instances (particularly after bright flashes) this activity consisted of an exaggerated, oscillatory discharge (Fig. 3*b*) in which the individual potentials were almost as large as the on transient of the PNR.

The PNR and the Amacrine Cell Response

As mentioned earlier, there is good evidence to support the hypothesis that the PNR reflects mainly amacrine cell activity, and the results in skate are consistent

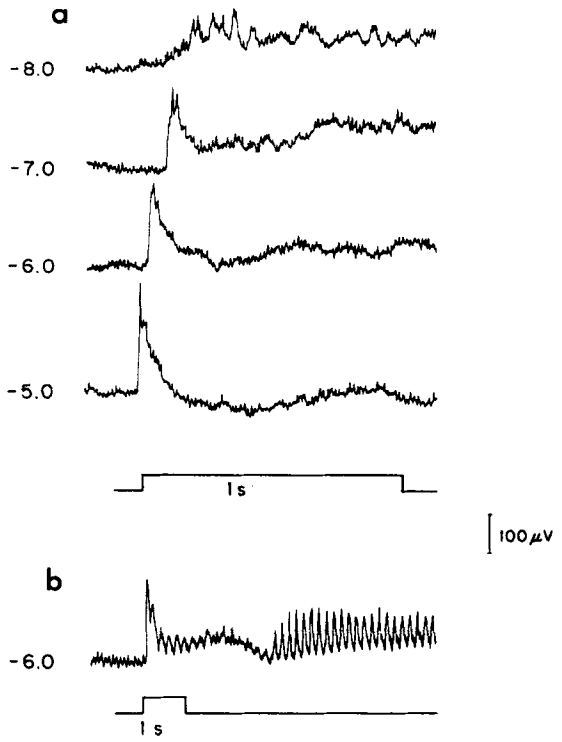

FIGURE 3. PNR recordings taken at a faster sweep speed (*a*) to illustrate in greater detail the features of the on transient. In (*b*) the PNR to log $I_t = -6$ contains a number of oscillatory potentials during and after photic stimulation.

with this view. We have on occasion obtained intracellular recordings from cells of the inner nuclear layer that were penetrated with fine (100–300 MΩ) pipettes filled with 2 M KCl or the fluorescent dye, procion yellow. Although a few of these elements were presumed on the basis of their response characteristics to be amacrine cells, only one was stained, subsequently recovered in histological section, and identified positively as an amacrine cell; its response to a 1-s flash at log $I_t = -5.0$ is shown in Fig. 4*a*. Large depolarizing potentials are seen after both the onset and the offset of the stimulus, and in each instance there is spike activity superimposed on the slower voltage swings. The presence of an "off"

response suggests that the retina was somewhat light adapted, since off transients were not usually observed in the intracellular records of fully dark-adapted preparations regardless of stimulus duration (Fig. 4*b*). Note also the absence of a sustained component in these records: i.e. the potentials begin to decay toward the base line before the stimulus has terminated. This is a variable feature of the intracellular response and has been found also in mudpuppy amacrine cells (Miller and Dacheux, 1976*b*).

The Effect of Tetrodotoxin (TTX) on the PNR

As shown in Fig. 4, the amacrine cell response to photic stimulation usually consisted of one or two spike potentials superimposed on a slower, transient

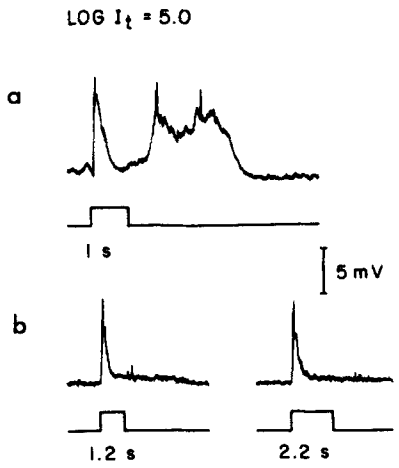

FIGURE 4. Intracellular recordings from amacrine cells in the skate retina elicited with full field stimulation. (*a*) The response of a partially light-adapted cell that was iontophoretically stained with procion yellow and subsequently identified in histological sections. (*b*) Recordings from a dark-adapted cell that responded with transient depolarizing potentials at the onset of light. The potentials exhibited the same features irrespective of stimulus duration.

depolarization (cf. Werblin and Dowling, 1969; Kaneko, 1970). Although the PNR probably reflects the nearly synchronous discharge of several such elements, it is not known whether the extracellular electrode detects mainly the slow transients or the spikes. One approach to this problem is through the application of tetrodotoxin, a substance which blocks the action potentials in a variety of nerve tissues by suppressing selectively the transient increase in sodium conductance induced by depolarization (Narahashi et al., 1964; Narahashi, 1974). The results of Figs. 5 and 6 indicate that neither the waveform nor the latency of the skate PNR are altered significantly by soaking the eyecup for 10 min in tetrodotoxin at 10^{-6} mg/ml, a concentration that blocks ganglion cell activity in the retina of the skate as well as in that of the frog (Murakami and Shigamatsu, 1970). In the frog the PNR also appears essentially unchanged by tetrodotoxin (Proenza and Ogden, personal communication; Karwoski, personal communication).

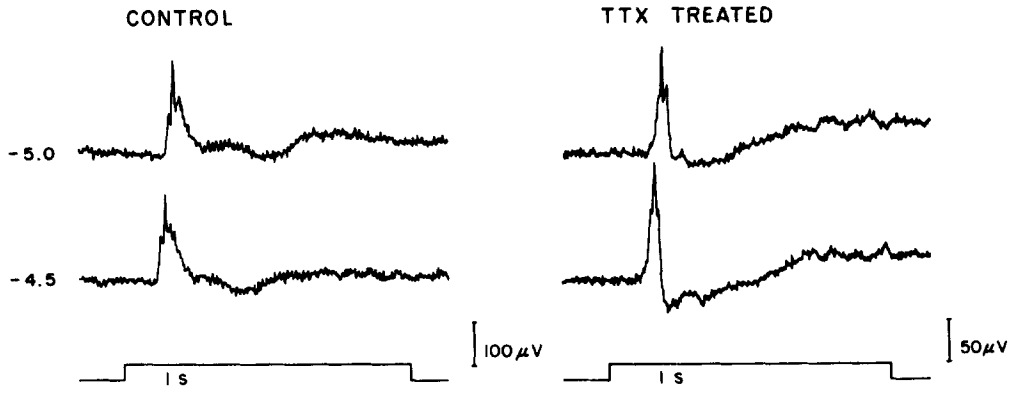

FIGURE 5. The effect of tetrodotoxin on the PNR. Neither the latency nor the slope of the rising phase of the on transient is affected by TTX. The falling phase of the response appears to begin earlier and to decline faster after TTX, but these changes are probably due to a slight shift in electrode position producing a more pronounced *b*-wave (note the undershoot of the base line with log $I_t = -4.5$). The amplitude of the response obtained from the TTX-treated retina was in this case slightly smaller than the control (note calibration markers).

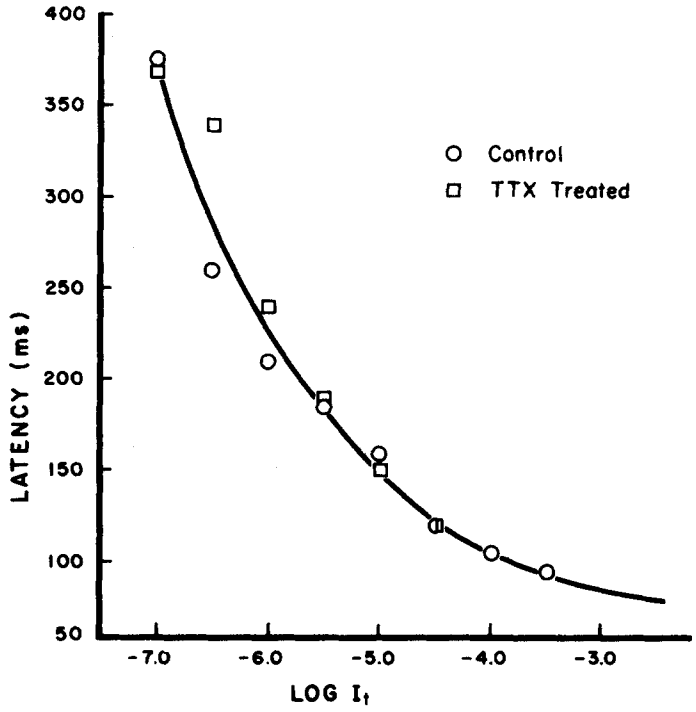

FIGURE 6. Latency data for the on transient of the PNR in control and TTX-treated retinas over a wide range of stimulus intensities. Latency refers to the interval between stimulus onset and the initiation of the response.

Light and Dark Adaptation

The changes in PNR threshold that were observed during both light and dark adaptation resemble those of the *b*-wave and ganglion cell discharge, especially with regard to the slow time course of the adaptive process (Green et al., 1975). In the experiments illustrated in Fig. 7, for example, the retinas were exposed to relatively dim background fields (log I_B = −7 and −6) and thresholds were determined before, during, and after the period of light adaptation. Despite the fact that these radiant exposures do not bleach a significant fraction of the

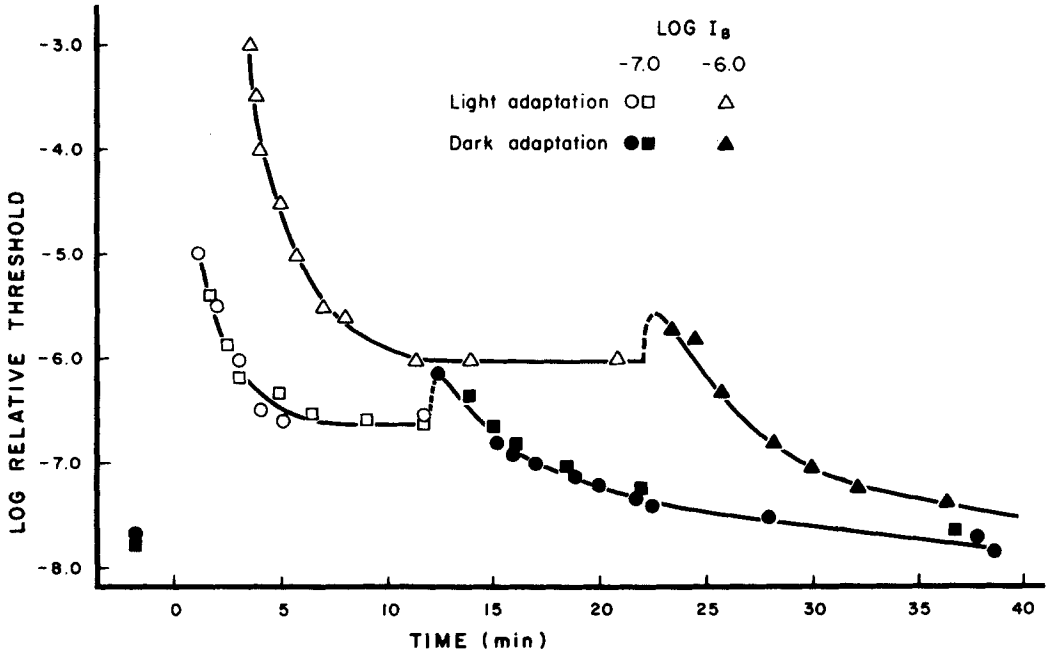

FIGURE 7. PNR thresholds during light (open symbols) and dark (filled symbols) adaptation. Although the light-adapting fields bleached insignificant amounts of rhodopsin, they induced relatively long silent periods in the light and a slow recovery of threshold in dark adaptation.

rhodopsin content of skate rods (Dowling and Ripps, 1971), there was in each instance a long "silent" period after the onset of the light; the brighter the adapting field, the longer the time during which the retina was refractory to photic stimulation (cf. Dowling and Ripps, 1970). When the PNR could again be elicited, the incremental threshold was several log units above its dark-adapted value, but it fell during the next 5–10 min to reach a plateau that was maintained for the remainder of the light-adapting period; it is steady-state levels such as these that give the increment threshold function of Fig. 10.

After the adapting field had been extinguished the recovery of sensitivity described an even slower time course. After a momentary rise in threshold, dark adaptation proceeded for about 25 min before the absolute (i.e. pre-exposure)

threshold was reached. Thus, in both light and dark adaptation the PNR exhibits temporal properties that are not grossly different from those that characterize other electrical responses of the proximal retina and which have been shown to be largely independent of changes of receptoral sensitivity (Green et al., 1975).

Light Adaptation and the Off Response

During the course of light adaptation, the waveform of the PNR changed markedly. The most striking aspect of this change was the emergence of an off response—a transient negative potential that followed the offset of the test stimulus. This is illustrated in Fig. 8, which shows responses elicited by a 250-μm test spot of fixed intensity (log $I_t = -3.5$) superimposed on a moderately bright background field (log $I_B = -5.0$); above each record is the time after the onset of I_B at which I_t was flashed. Since the exposure to I_B induced initially a silent period lasting more than 6 min, the first recordable response occurred after 7

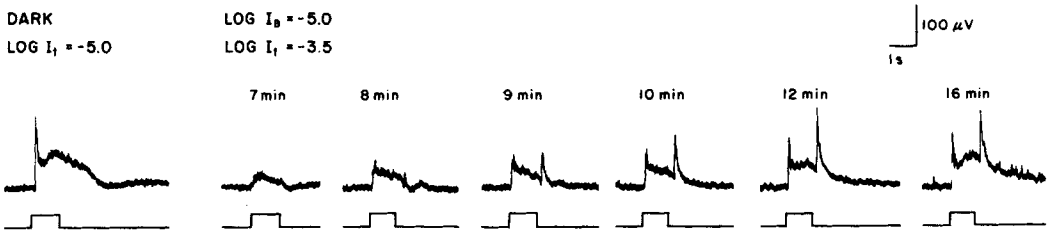

FIGURE 8. The development of an off transient in the PNR during the course of light adaptation. Responses to a constant intensity flash (log $I_t = -3.5$) were elicited at various times after the onset of a background field (log $I_B = -5.0$). The test flash was about 2 log units above the light-adapted threshold. For comparison, the response of the dark-adapted preparation to a test flash 2.5 log units above threshold is shown at the left.

min of light adaptation. At that time, the PNR was only slightly above threshold amplitude but already contained a small off transient. With further light adaptation, both the on and the off components grew in amplitude, became more sharply peaked as their latencies shortened, and finally exhibited very similar transient potentials. Similar changes in the shape of the PNR during light adaptation have been observed in mudpuppy (Proenza and Burkhardt, 1973).

We noted earlier that the amplitude of the on transient in the dark-adapted retina is affected by the diameter of the test stimulus; this is true also of the light-adapted preparation. However, spot size usually had opposite effects on the two components of the light-adapted PNR. Thus, with the 250-μm stimulus spot, the amplitude of the off response either equalled or slightly exceeded that of the on transient (Fig. 8). But as shown in Fig. 9, the situation could be reversed by reducing the test spot to a diameter of 100 μm, or exaggerated by enlarging the stimulus field to 500 μm; the latter greatly enhanced the off response and severely depressed the on component. This phenomenon has also been observed in the mudpuppy PNR (Proenza, personal communication).

The Increment Threshold

The effect of background illumination on the incremental threshold (i.e. the steady state plateau in light adaptation) is shown in Fig. 10. The incremental threshold function for the PNR usually displays, as in Fig. 10, two distinct slopes.

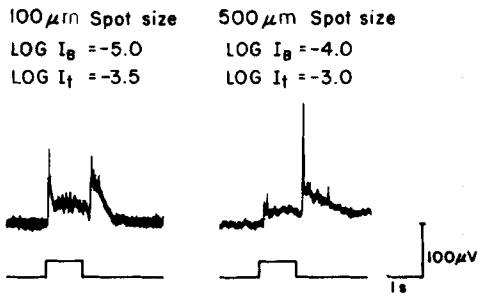

FIGURE 9. The enhancement of the off component of the PNR by increasing the diameter of the test spot.

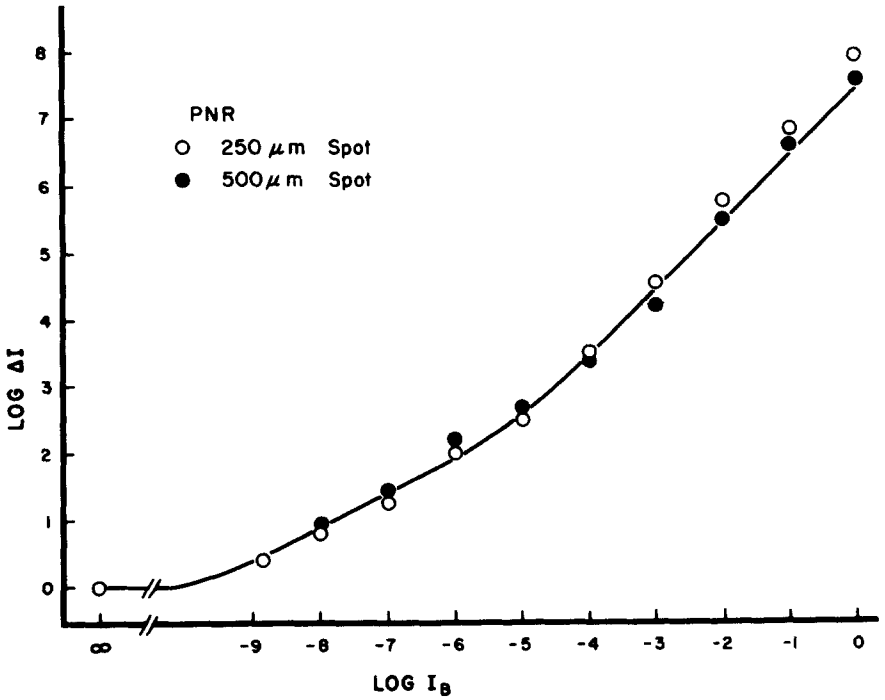

FIGURE 10. Increment-threshold data for the on component of the PNR obtained with 250- and 500-μm test spots superimposed on background fields that fully illuminated the retina. The ordinate values are graphed relative to the threshold level of the dark-adapted retina. See text for details.

Over the first 4 log units of background intensity (log I_B = −9.0 to −5.0), the slope of the function is about 0.6. As the background level is raised above log I_B = −5.0, the PNR incremental threshold function becomes steeper and adheres closely to the unit slope of the Weber-Fechner relationship, $\Delta I = I_B \times$ constant.

The shape of the PNR increment threshold function and its position on the scale of I_B appear to be quite independent of stimulus size and duration. We found similar PNR increment threshold functions with stimulus spots varying between 100 and 1,000 μm in diameter and with stimulus durations ranging between 0.2 and 1 s. Moreover, even after application of TTX, the increment threshold results were similar to those illustrated in Fig. 10.

We have previously reported that responses of the proximal retina in the skate adhere closely to the Weber-Fechner relationship over most of their adaptive range (Dowling and Ripps, 1970; Green et al., 1975). A re-examination of incremental threshold functions for both the *b*-wave and ganglion cell discharge in the skate revealed considerable variability in slope of the function over the first four log units of background intensity. This variability appeared to be related to the absolute threshold of the dark-adapted retina; the higher the initial threshold, the flatter was the initial slope of the incremental threshold function. The slopes we obtained ranged from 0.3 to 0.9, but most often the initial slope was between 0.5 and 0.7. However, regardless of its slope over the first 4 log units of background intensity, the incremental threshold function always assumed a slope of unity with higher background intensities (i.e. $\log I_B > -5$).

Fig. 11 shows some selected results obtained from incremental threshold runs on the *b*-wave, ganglion cell discharge, and PNR of the skate. In all three cases the initial slope of the function was 0.5–0.6, and the incremental thresholds for the three types of responses were very similar over the entire adaptive range. Also shown in the figure is the curve resulting from threshold measurements of the receptor potential and horizontal cells for equivalent adapting conditions (Green et al., 1975). The fact that this curve is shifted by about 2 log units to the right on the scale of I_B indicates that the PNR, *b*-wave, and ganglion cell thresholds are 100 times more sensitive to ambient illumination than are those of the receptor potential or horizontal cells. In addition, the incremental threshold function for the receptors and horizontal cells has a slope of one over nearly the entire range of I_B. Our observations indicate, therefore, that the incremental threshold functions for the PNR, *b*-wave, and ganglion are quite similar, and that they are distinctly different from the incremental threshold functions for the receptors and horizontal cells. It is noteworthy that the incremental threshold function for *all* retinal responses in skate rises with unit slope above $\log I_B = -5$ (see Discussion).

The b-Wave and the PNR

The fact that the incremental threshold functions for the PNR, *b*-wave, and ganglion cell discharge exhibit considerable variability prompted a closer examination of the behavior of the *b*-wave and PNR during light and dark adaptation. Fig. 12 shows the results obtained during and after exposure to the same adapting intensity ($\log I_B = -5.0$); the absolute thresholds for the two responses were nearly the same and are plotted at the left of the figure. After the onset of the adapting field, neither the *b*-wave nor the PNR could be elicited for about 7 min, although the silent period for the receptor potential (i.e. the *a*-wave) lasted only 1 min. We invariably found that in the course of light adaptation the *b*-wave reached the plateau of its threshold recovery before the PNR had fully light adapted. However, the temporal differences were quite variable and depended

largely upon the intensity of the adapting field; the lower the level of I_B, the smaller were the differences in the silent period and in the time required to reach the plateau of light adaptation.

Of interest was the finding that, despite its late onset, the PNR function often crossed that of the *b*-wave and consequently the PNR incremental threshold fell below that of the *b*-wave. However, any threshold differences between

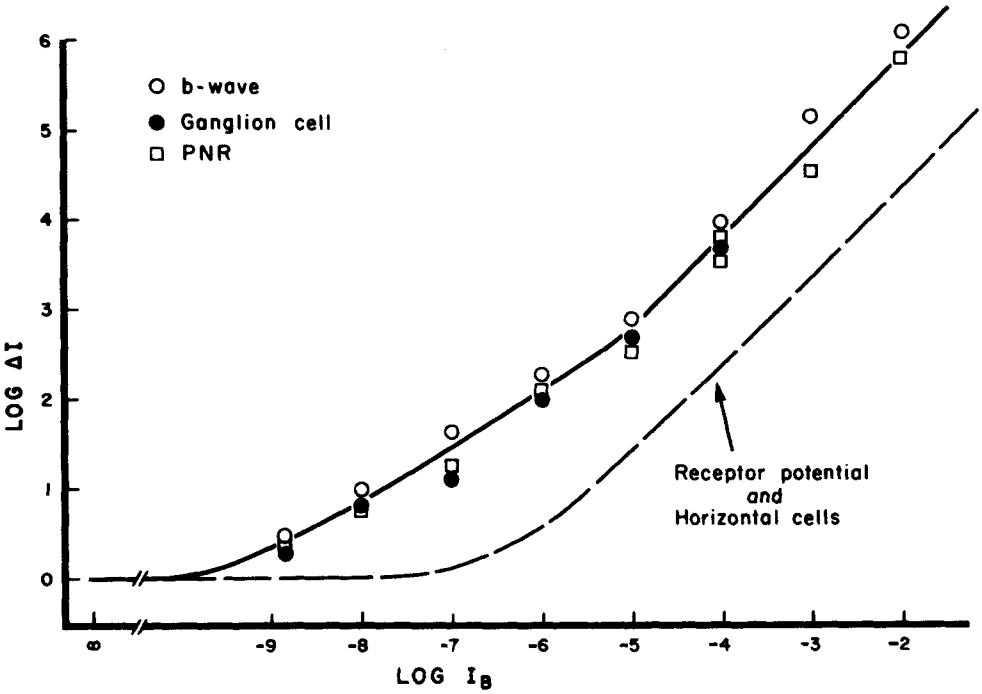

FIGURE 11. Increment threshold functions for the *b*-wave, ganglion cell discharge, and PNR. The data are from single, representative experiments. The *b*-wave and ganglion cell responses were elicited with full field stimulation, the PNR with a 250-μm spot. Threshold response criteria for the *b*-wave was 10 μV; for the ganglion cell discharge, one or two spikes. The dashed line illustrates receptor potential results obtained in an earlier study (Green et al., 1975). See text for details.

the *b*-wave and the PNR in the light-adapted retina were erased in dark adaptation, due initially to the marked effect on PNR sensitivity of turning off the background field. In Fig. 12, for example, the PNR threshold of the light-adapted retina was about 0.4 log unit less than the *b*-wave plateau. But when the adapting light was extinguished, the PNR threshold rose at first to the level of the *b*-wave, and thereafter the two responses dark adapted along the same time course.

The convergence of the two threshold measures in dark adaptation could be demonstrated also with a somewhat different experimental protocol, one in which PNR and *b*-wave thresholds were measured alternately in the same run.

For the experiments illustrated in Fig. 13, more intense adapting fields were used (log I_B = −2.0 and −1.0), but the duration of light adaptation was shorter (10 s). In both runs the background was extinguished during the silent periods, and incremental thresholds could not be measured. In fact, the refractoriness continued into dark adaptation and, as was the case during light adaptation (Fig. 12), the *b*-wave response returned earlier than the PNR. However, once the PNR appeared, its threshold fell rapidly to meet the curve of *b*-wave adaptation, and

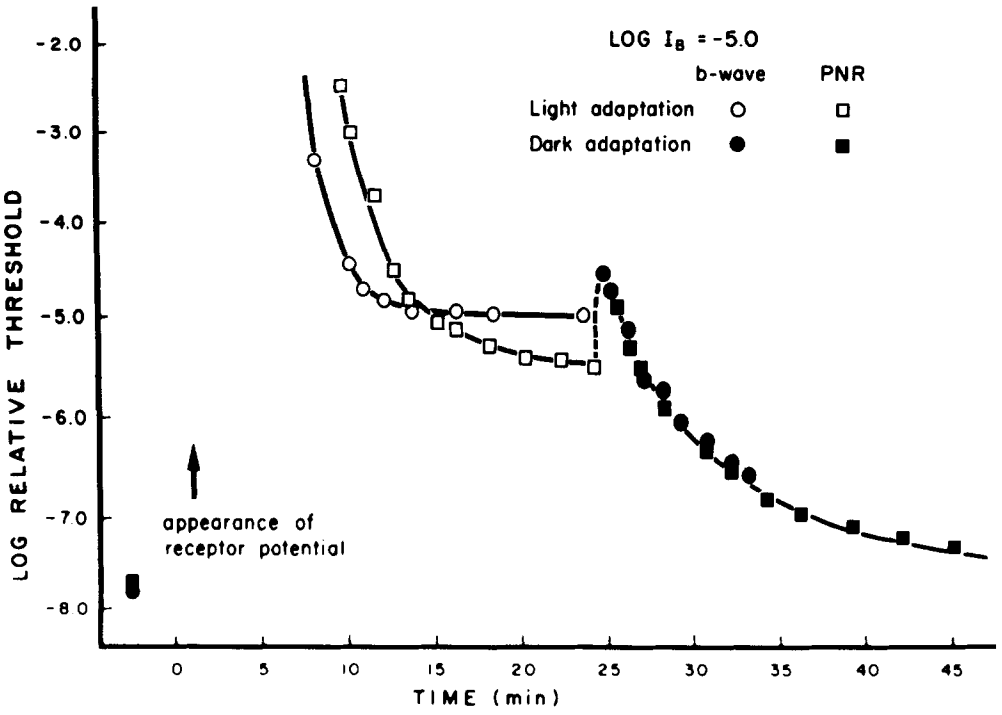

FIGURE 12. Threshold measurements on the PNR and *b*-wave obtained from responses recorded during light and dark adaptation. The relatively bright light-adapting field induced prolonged silent periods for these potentials as compared with that of the receptor potential (arrow). See text for details.

thereafter the two responses adapted in concert. Note also that the general features described above apply to both sets of data, despite the fact that the weaker adapting field did not bleach a significant fraction of the available rhodopsin, whereas the 10-s exposure to log I_B = −1.0 bleached about 25% of the rhodopsin content of the rods (Dowling and Ripps, 1971).

DISCUSSION

The results of this study are consistent with the notion that the PNR of the skate retina is derived primarily from the activity of amacrine cells. The transient components of the PNR resembled those obtained by intracellular recording

from amacrine cells, and the amplitude of the extracellular response was maximum in the proximal region of the inner nuclear layer where the perikarya of the amacrine cells are located. In addition, the large oscillatory potentials that were seen occasionally during and after photic stimulation are believed to arise from elements of the inner nuclear or inner plexiform layers of the retina (Yonemura et al., 1963; Doty and Kimura, 1963; Ogden, 1973). Recent evidence that glycine induces reversible amacrine cell lesions, together with a transient loss of the oscillatory potentials, suggests that the amacrine cell may be the source of these oscillations (Korol et al., 1975).

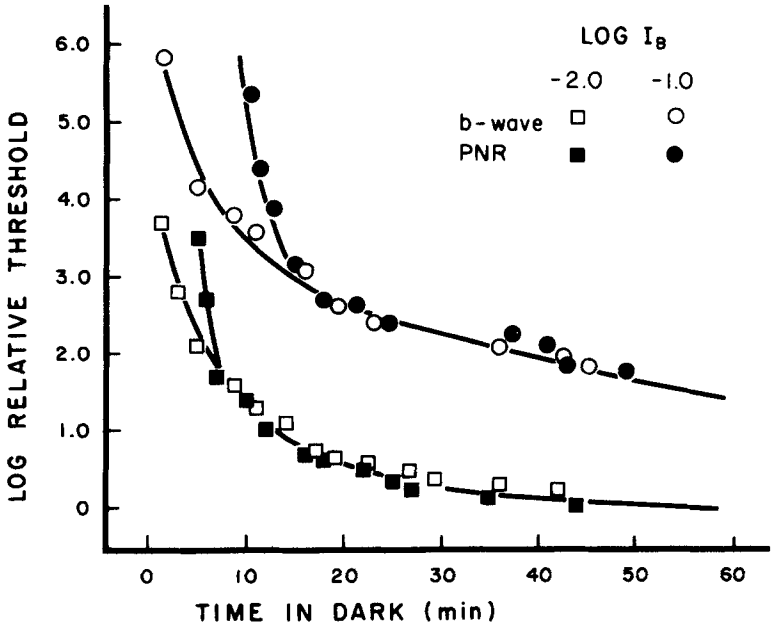

FIGURE 13. Dark adaptation of the *b*-wave and PNR after 1-s exposures to intense light-adapting fields. PNR and *b*-wave thresholds for each exposure condition were determined in the same experimental run. Note that although the two potentials became responsive at different times after the light was extinguished, the thresholds merged and ran together in dark adaptation.

With regard to the waveform of the PNR, our finding that the potential is relatively unchanged by the application of tetrodotoxin suggests that the spike activity generated by amacrine cells (Werblin and Dowling, 1969; Kaneko, 1970) does not contribute significantly to the extracellularly recorded response. Although tetrodotoxin-resistant action potentials occur in cells in which Na^+ is not the principal current carrier (Kao, 1966; Zipser and Bennett, 1973), or in those containing Na^+ channels that are insensitive to TTX (Narahashi, 1974; Kleinhaus and Prichard, 1976), the studies of Miller and Dacheux (1976*b*) have shown that both the soma and dendrites of amacrine cells generate impulses that are suppressed by TTX. Thus, it appears likely that the PNR reflects mainly the graded, nonregenerative components of the amacrine cell response.

The adaptive properties of the PNR were a primary concern of the present experiments, and our findings indicate that in most respects that PNR behaved like other responses arising proximally within the skate retina (Dowling and Ripps, 1970, 1971, 1972; Green et al., 1975). The onset of a steady background field induced a silent period of relatively long duration; very low levels of I_B affected markedly the sensitivity of the PNR to incremental stimuli (Fig. 10); and in dark adaptation, PNR thresholds followed a slow time course after exposure to relatively dim adapting fields that bleached only trivial amounts of visual pigment (Fig. 7). Thus the PNR exhibits many properties of network adaptation.

There were, however, some small but rather consistent differences between the adaptive properties of the PNR and the *b*-wave. After the onset of a moderately bright adapting field or after the extinction of a short adapting exposure, *b*-wave responses inevitably appeared before the PNR, although with time the thresholds for both responses converged. To what extent these discrepancies reflect a difference in the reaction of these two responses to the processes underlying network adaptation is unclear.

The differences between the adaptive properties of the *b*-wave and the PNR are very small, however, when compared to the differences between the adaptive properties of the responses arising distally in the retina (i.e. receptor potential and horizontal cell response) and those arising more proximally in the retina (i.e. *b*-wave, PNR, and ganglion cell discharge). Receptor potentials reappear very rapidly after the onset of an adapting field as compared with the *b*-wave or PNR (Fig. 12), the time course of both light and dark adaptation for the receptor potential is very fast relative to that exhibited by the *b*-wave, PNR, or ganglion cell discharge (compare Fig. 7 with Figs. 7 and 8 in Green et al., 1975), and the incremental threshold function for the receptor and horizontal cell potential is displaced by about two logs units on the scale of I_B relative to that obtained from measurements of the PNR, *b*-wave, and ganglion cell discharge (Fig. 11). The present results, therefore, confirm and extend our earlier findings that responses arising proximally in the skate retina have adaptive properties distinctly different from distal responses, and that important adaptive processes take place within the neuronal network of the inner retina (Green et al., 1975).

As yet the mechanisms underlying network adaptation have not been identified. However, the remarkably slow time course of network adaptation has led to the speculation that cells of the proximal retina are influenced by alterations in the concentration of a desensitizing substance that accumulates within the neuronal network during light exposure. In this regard, we have found that increased levels of extracellular potassium, which exert little or no effect on the thresholds of the receptor potential or horizontal cell response, can desensitize the *b*-wave and PNR of the skate retina (Dowling and Ripps, 1976, and manuscript in preparation). Some of the variability in both the absolute thresholds and the initial slopes of the increment threshold functions of responses arising in the proximal retina may be related to this factor. We noted, for example, that the higher the dark-adapted threshold of a particular response, the less sensitive was the response to background illumination. A number of recent

studies have shown that ongoing electrical activity as well as metabolic failure due to anoxia or other causes may result in a variable release of K^+ from neuronal elements (Vyskocil et al., 1972; Moody et al., 1974; Lotham and Somjen, 1975). It may be that responses exhibiting lower absolute sensitivities are from retinas which contain higher levels of a desensitizing agent such as potassium.

The variability in the incremental threshold function exhibited by the responses arising proximally in the skate retina largely disappears at the brighter backgrounds, i.e. above log $I_B = -5$. It is at these background levels that the receptor incremental threshold function rises with a slope of unity (Fig. 11). It seems likely, therefore, that the receptors govern the light adaptation process for the entire retina at these brighter backgrounds (Green et al., 1975; Dowling and Ripps, manuscript in preparation).

In summary, our results are consistent with the view that the PNR reflects mainly amacrine cell activity and that amacrine cells show adaptive properties similar to those of other cells of the proximal retina. The role of the amacrine cells in retinal function is as yet unclear, although they are believed to provide a major input to the "on-off"-type ganglion cell (Werblin and Dowling, 1969; Werblin and Copenhagen, 1974). Indeed, the spike frequency of this class of ganglion cell appears to be a linear function of the peak amplitude of the PNR transient (Burkhardt and Whittle, 1973), and the two electrical responses correlate well with regard to threshold sensitivity (Burkhardt, 1970), receptive field size, and area dependence (Holden, 1972). However, the extent to which amacrine cells feed back onto bipolar cells or provide input to other types of ganglion cells is still uncertain (cf. Naka, 1976), as is whether amacrine cell effects are primarily excitatory (Dowling and Werblin, 1969; Burkhardt and Whittle, 1973) or inhibitory (Werblin and Copenhagen, 1974; Miller and Dacheux, 1976*a*). But regardless of which alternatives prove to be the case, our results indicate that the adaptive properties of the amacrine and ganglion cells and the *b*-wave in the skate retina during and after steady light adaptation are quite similar.

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REFERENCES

BURKHARDT, D. A. 1969. Distinction between a proximal negative response and the local b-wave in the retina. *Nature (Lond.).* **221**:879–880.

BURKHARDT, D. A. 1970. Proximal negative response of frog retina. *J. Neurophysiol.* **33**:405–420.

BURKHARDT, D. A., and P. WHITTLE. 1973. Intensity coding in the frog retina. Quantitative relations between impulse and graded activity. *J. Gen. Physiol.* **61**:305–322.

CAVANAUGH, G. M. 1975. Formulae and Methods (VI) of the Marine Biological Laboratory Chemical Room. Marine Biological Laboratory, Woods Hole, Mass. 70.

DOTY, R. W., and D. S. KIMURA. 1963. Oscillatory potentials in the visual system of cats and monkeys. *J. Physiol. (Lond.).* **168**:205–218.

DOWLING, J. E., and B. B. BOYCOTT. 1966. Organization of the primate retina: electron microscopy. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **166**:80–111.

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.

DOWLING, J. E., and H. RIPPS. 1976. Potassium and retinal sensitivity. *Brain Res.* **107**:617–622.

GREEN, D. G., J. E. DOWLING, I. M. SIEGEL, and H. RIPPS. 1975. Retinal mechanisms of visual adaptation in the skate. *J. Gen. Physiol.* **65**:483–502.

HOLDEN, A. L. 1972. Proximal negative response in the pigeon retina. *J. Physiol. (Lond.).* **221**:173–188.

KANEKO, A. 1970. Physiological and morphological identification of horizontal, bipolar and amacrine cells in goldfish retina. *J. Physiol. (Lond.).* **207**:623–633.

KAO, C. Y. 1966. Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. *Pharmacol. Rev.* **18**:977–1099.

KLEINHAUS, A. L. and J. W. PRICHARD. 1976. Sodium dependent tetrodotoxin-resistant action potentials in a leech neuron. *Brain Res.* **102**:368–373.

KOROL, S., P. M. LEUENBERGER, U. ENGLERT, and J. BABEL. 1975. *In vivo* effects of glycine on retinal ultrastructure and averaged electroretinogram. *Brain Res.* **97**:235–251.

LOTHAM, E. W., and G. G. SOMJEN. 1975. Extracellular potassium activity, intracellular and extracellular potential responses in the spinal cord. *J. Physiol. (Lond.).* **252**:115–136.

MILLER, R. F., and R. DACHEUX. 1976*a*. Synaptic organization and ionic basis of on and off channels in mudpuppy retina. III. A model of ganglion cell receptive field organization based on chloride-free experiments. *J. Gen. Physiol.* **67**:679–690.

MILLER, R. F., and R. DACHEUX. 1976*b*. Dendritic and somatic spikes in mudpuppy amacrine cells: identification and TTX sensitivity. *Brain Res.* **104**:157–162.

MOODY, W. J., JR., K. J. FUTAMACHI, and D. A. PRINCE. 1974. Extracellular potassium activity during epileptogenesis. *J. Exp. Neurol.* **42**:248–263.

MURAKAMI, M., and Y. SHIGEMATSU. 1970. Duality of conduction mechanism in bipolar cells of the frog retina. *Vision Res.* **10**:1–10.

NAKA, K. I. 1976. Neuronal circuitry in the catfish retina. *Invest. Ophthalmol.* **15**:926–935.

NARAHASHI, T. 1974. Chemicals as tools in the study of excitable membranes. *Physiol. Rev.* **54**:813–889.

NARAHASHI, T., J. W. MOORE, and W. R. SCOTT. 1964. Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. *J. Gen. Physiol.* **47**:965–974.

OGDEN, T. E. 1973. The proximal negative response of the primate retina. *Vision Res.* **13**:797–807.

PROENZA, L. M., and D. A. BURKHARDT. 1973. Proximal negative response and retinal sensitivity in the mudpuppy, *Necturus maculosus*. *J. Neurophysiol.* **36**:502–518.

PROENZA, L. M., and R. E. MORTON. 1975. A device for beveling fine micropipettes. *Physiol. Behav.* **14**:511–513.

VYSKOCIL, F., N. KRIZ, and J. BURES. 1972. Potassium selective microelectrodes used for measuring the extracellular brain potassium during spreading depression and anoxic depolarization in rats. *Brain Res.* **39**:255–259.

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 retina of the mudpuppy, *Necturus maculosus*. II. Intracellular recording. *J. Neurophysiol.* **32**:339–355.

YONEMURA, D., Y. MASUDA, and M. HATTA. 1963. The oscillatory potential in the electroretinogram. *Jpn. J. Physiol.* **13**:129–137.

ZIPSER, B., and M. V. L. BENNETT. 1973. Tetrodotoxin resistant electrically excitable responses of receptor cells. *Brain Res.* **62**:253–259.