Control of Retinal Sensitivity

III. *Lateral Interactions at the Inner Plexiform Layer*

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ABSTRACT Both the "on" and the "on-off" ganglion cells in the mudpuppy retina generate graded responses over a narrow range of log test intensities. Sustained full field or surround backgrounds change the range of center log test intensities that elicits the graded response for both cell types. The on-off, but not the on ganglion cells are further affected by moving or flashing surround backgrounds. These cells are hyperpolarized, threshold is elevated, and the entire graded range of response is elicited by a higher range of log center test intensities. Depolarizing activity is elicited in amacrine cells by moving backgrounds that affect the on-off ganglion cells, but bipolar activity is unaffected. These results suggest that the amacrine cells at the inner plexiform layer mediate a third stage of sensitivity control in the retina, increasing threshold for response to change specifically in the on-off ganglion cells.

INTRODUCTION

In this study we have characterized the effects of lateral interactions at the inner plexiform layer, mediated by the amacrine cell system, upon the signal conveyed from bipolar to ganglion cells. The work is predicated on earlier results indicating that the amacrine cells respond transiently to either flashing (Werblin and Dowling, 1969) or moving (Werblin, 1970) stimuli, and that a spinning "windmill" pattern elicits depolarizing activity in the amacrine cells over a broad region of the retina (Werblin, 1972). Anatomical studies show that amacrine cells have processes that extend laterally over at least a few hundred microns in *Necturus* (Dowling and Werblin, 1969), and this is characteristic of amacrine cells in a variety of vertebrate retinas (Cajal, 1972). The transient form of amacrine cell activity seen in *Necturus* has also been observed in fish (Kaneko, 1971) and frog (Matsumoto and Naka, 1972), although these animals appear to have a more sustained form of amacrine cell

response as well. These studies suggest that an amacrine cell system, extending laterally across the inner plexiform layer and responding transiently to change in stimulus may be a general characteristic of vertebrate retinas.

The functional properties of the amacrine cell system can be inferred from physiological studies in retinas that have been shown to have elaborate inner plexiform layers with an abundance of amacrine-to-amacrine and amacrine-to-ganglion cell "conventional" synapses (Dubin, 1970). In frog (Maturana et al., 1960), pigeon (Maturana and Frenk, 1963), rabbit (Barlow and Levick, 1965), and ground squirrel (Michael, 1968) a major class of ganglion cell responds best to movement, often in a particular direction across the receptive field. Barlow and Levick (1965) have suggested that the directional specificity of ganglion cells in rabbit is mediated by a form of movement-elicited lateral inhibition. Most of the movement-sensitive ganglion cells have receptive fields with movement-sensitive surrounds that antagonize the response to movement through the center of the field, suggesting a further role for movement-sensitive lateral interneurons.

Psychophysical experiments show that visual threshold is dramatically elevated for the first few hundred milliseconds following the presentation of a background field (Crawford, 1947). Teller et al. (1971) have shown that flashed annular backgrounds also elevate threshold for a test target at their centers, and that the effect is greatly diminished under dichoptic conditions. These experiments suggest that threshold can be partly elevated by a change-sensitive system of lateral interneurons in the retina.

In our experiments we have utilized flashing and moving stimuli that elicit lateral interactions specifically from the change-sensitive amacrine cell system, and we have studied the effects of the amacrine cells upon the response properties and sensitivity of the ganglion cells. The results suggest that only the activity of the on-off ganglion cells is antagonized by the amacrine cell system; the on ganglion cells are unaffected by interactions at the inner plexiform layer.

METHODS

Extracellular Electrodes

The preparation, stimulator, and recording apparatus used in these experiments were similar to those used in the two accompanying studies, with one exception. In order to record extracellular activity ganglion cells in mudpuppy we used Teflon coated platinum iridium wire 25 μm in diameter (Medwire Corp., Mount Vernon, N. Y.) as used in rabbit by Ames and Pollen (1969). The teflon was stripped from one end of a short (1-cm) length of the wire and this end was soldered to a thicker silver lead that connected with the recording apparatus. The other end of the teflon coated wire was cut with a sharp razor blade so that the end surface was presumably flat, and this was then lowered to the retinal surface for recordings. The vitreous was drained away with

a 1-mm capillary tube after the anterior eye was removed. After about ½ h the surface of the retina appeared to be relatively dry, and single units could be easily isolated and studied for more than 1 h. On-off ganglion cells in mudpuppy respond with brief burst usually consisting of less than 10 impulses. These were counted by eye on the face of a storage oscilloscope. In most experiments the total number of spikes at "on" or "off" in the ganglion cells studied did not exceed five, and the number of spikes in each trial was usually constant. Therefore, although we "averaged" responses over three or more trials, the average number of spikes was often an integral number.

Stimulus Intensity

Adaptation to background levels in Figs. 2, 5, and 12 was measured with a stimulator that was calibrated with the intensity levels used in the previous studies (Normann and Werblin, 1974; Werblin, 1974) so the log relative threshold can be compared for all cell types. This stimulator presented test intensities in steps of 0.2 or 0.5 log units, so some of the initial points on the intensity-response curves for smaller increments in the ganglion and amacrine cells were not measured. However, our intent was to show that the response curves for these cells behave roughly like those for the more distal cells under different background conditions, so the data are sufficient. In other experiments where we describe the effect of lateral interaction on graded response, such as Figs. 8, 10, and 11, the oscilloscope stimulator described previously was used (Werblin, 1973).

In most of these experiments we have attempted to describe the effect of lateral antagonism in the receptive field the ganglion cells elicited by flashing spots, flashing annuli, and by moving vanes of a "windmill" pattern presented to the periphery of the receptive field. We have chosen a specific region in the periphery at which to present these stimuli, as shown in Fig. 1. The inside diameter of the annulus and the windmill vanes, both centered upon the test field, was 1 mm, and the spot was flashed at about 500 μm from the center of the test field. Therefore all lateral effects were

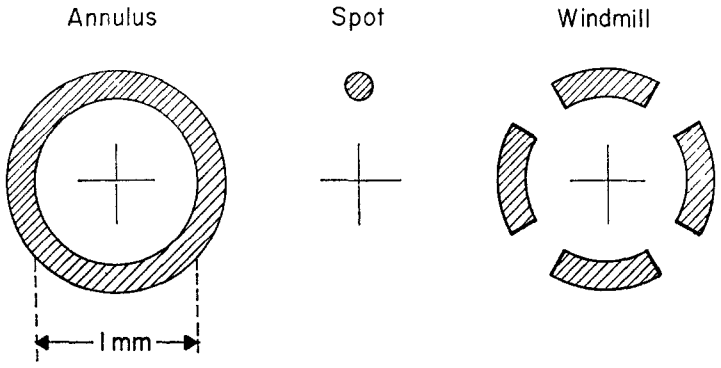

FIGURE 1. Background illumination configurations. The three backgrounds and full fields were used exclusively throughout this paper. They fall ½ mm from the center of the test field. Test stimuli, 300 μm in diameter, presented at the centers, were used to evaluate the response characteristics of cells in the presence of these backgrounds.

initiated by stimuli 500 μm from the center of the receptive field of the ganglion cell. This distance and the configuration of background does not seem to be critical: Copenhagen (1972) had shown that the effects are qualitatively the same for a variety of peripheral stimulus conditions.

RESULTS

Amacrine and ganglion cells both seem to generate action potentials associated with transient responses at on and off. It is therefore important to distinguish between the two cell types in these studies. In intracellular studies of the mudpuppy, the amacrine cell can be distinguished by its single spike at each transient, accompanied by a relatively large sharply peaked transient depolarization (Werblin, 1970). The ganglion cells usually show a greater number of spikes and a relatively smaller depolarization (Werblin and Dowling, 1969). This difference in response form is not as clear in other species such as frog (Matsumoto and Naka, 1972) or goldfish (Kaneko, 1971) where amacrine cells seem to generate many spikes with each transient. Occasionally identification is equivocal, particularly when the amacrine cells are damaged, so studies were carried out only in clearly distinguishable cases. Our extracellular recording methods preclude retinal penetration, so these data are probably taken from ganglion cells and correlate well with the intracellular data taken from the presumed ganglion cells.

Effects of Full Field Backgrounds

We recorded the responses of the on-off ganglion cells to diffuse flashes of different intensities at several background levels, counted the total number of spikes elicited by the flashes, and plotted the intensity-response curves. A typical result for the on component of an on-off ganglion cell is shown in Fig. 2. These response curves are graded over a narrow range of intensities, roughly 1 log unit, and shift parallel to each other as background level is changed. The shifting phenomenon is characteristic of the bipolars described in the accompanying paper (Werblin, 1974). These curves, compared with those of the bipolars, suggest that a spike can be elicited in ganglion cells with test stimuli that polarize bipolars less than 100 μV. The results are consistent with those of Barlow and Levick (1970) in the cat and of Byzov and Kusnezova (1971) in frog who also showed curves that shifted with background level although those curves became steeper at higher background intensities.

As Fig. 3 illustrates, the transient response to diffuse test flashes recorded intracellularly in the on-off ganglion cell results from the near superposition of depolarizing and hyperpolarizing transients which tend to increase in magnitude and decrease in latency as test flash intensity is raised. Each of the antagonistic components is initiated at a different site in the ganglion cell receptive field, and can be elicited separately by distinct forms of stimulus.

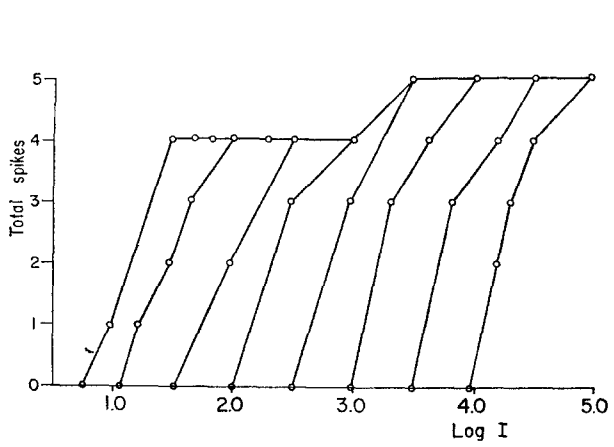

FIGURE 2.

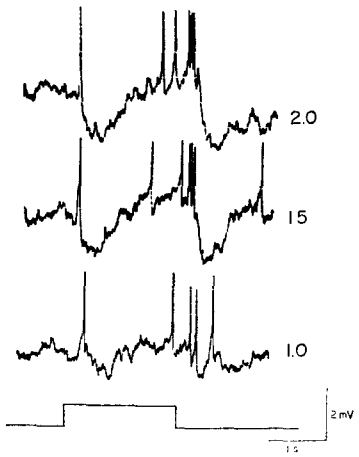

FIGURE 3

FIGURE 2. Intensity-response curves of the on-component for an on-off ganglion cell at different backgrounds. The resolution of the stimulator was limited to 0.2 log units, so there are few points between threshold and maximum response. Each point is the average of three measurements. Abscissa is log relative full field test flash intensity. 1-s test flashes were presented in ordered sequence at 9-s intervals.

FIGURE 3. Intracellular recording of on-off ganglion cell to diffuse test flashes increase in depolarizing and hyperpolarizing components at both on and off, but little increase in spike output with increasing test flash intensity. The log 1.0 test flash was near threshold, the log 2.0 test flash elicited the maximum number of spikes possible from this cell. Spike height is attenuated by the low band pass of the recording system.

Separation of the Depolarizing and Hyperpolarizing Transient Components

Each antagonistic component in the ganglion cell response was selectively activated by stimulating either the center or the surround of the receptive field as shown in Fig. 4. Fig. 4 A shows that mostly depolarizing transients, associated with spike activity, were elicited by a central flash, 300 μm in diameter, while mostly hyperpolarizing activity with no spikes at on was elicited by an annulus of the same intensity, 1 mm in inside diameter, 200 μm wide, and centered on the receptive field. The residual hyperpolarizing transient, seen even in response to the central flash, is probably due to a surround that extended through the center of the receptive field, or to scatter from the central flash into the surround.

A spot, flashed in the periphery of the receptive field, elicits a transient hyperpolarization which, when properly timed can compete with and eliminate the central response. Fig. 4 B shows that when the peripheral spot was flashed 400 ms before the central flash it caused a small transient hyperpolarization separate from the response to the central flash, but when the peripheral spot was flashed 250 ms before the central flash it reduced the depolarization at on and eliminated the spike activity in the central response. This is an example of interactions between center and surround in the receptive field of

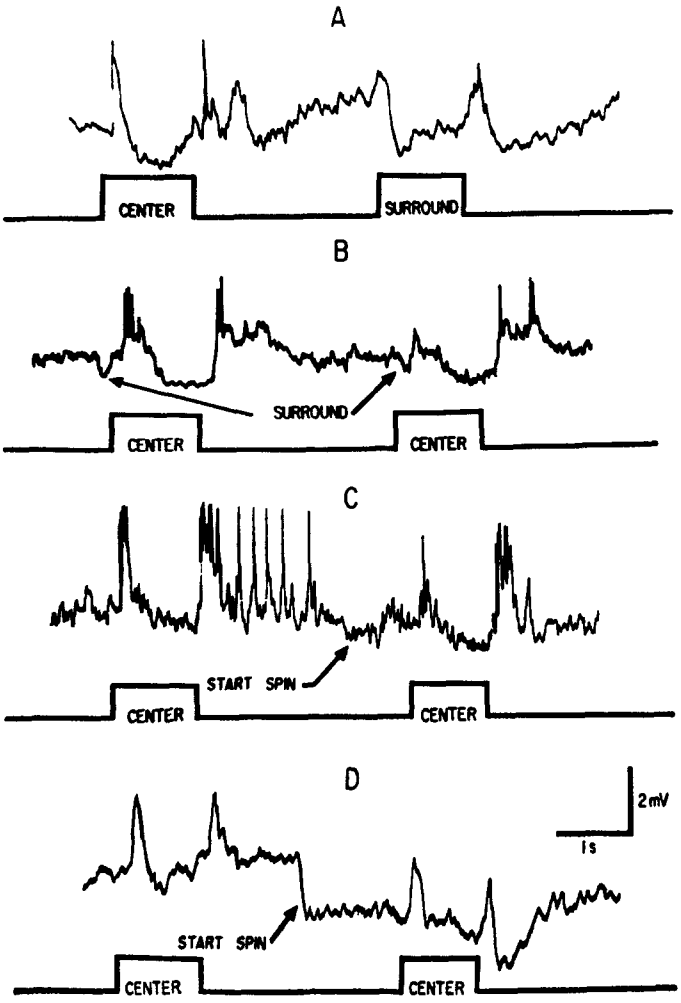

FIGURE 4. Intracellular recording of antagonistic components of the on-off ganglion cell response. (A) Response of an on-off ganglion cell to a central spot 300 μm microns in diameter (left) and to annulus, 1 mm in diameter, 300 μm microns wide, of same intensity (right). The response is mostly depolarizing to central illumination; mostly hyperpolarizing to surround illumination. (B) Response of on-off ganglion cell to central test spot preceded by flash of peripheral spot about 400 ms (left) and 250 ms (right). The peripheral spot, when properly timed, can act to eliminate the centrally evoked impulse response (right). (C) Effect of the windmill on the response of an on-off ganglion cell to the central flash. Left: response in presence of stationary windmill. Right: response in presence of the spinning windmill. The membrane is slightly hyperpolarized for the duration of the spin, the spontaneous activity is eliminated, and the response to the central flash is diminished. (D) Effect of the windmill on a depolarized ganglion cell. Depolarization of about 4 mV was monitored by the recording electrode that had probably damaged the cell. The effect of the windmill on the ganglion cell membrane here is more dramatic, showing a larger hyperpolarization during the spin. Spikes have been blocked by the depolarization, but depolarizing transients are still elicited at on and off.

the on-off ganglion cell. It shows that activity, elicited by change, i.e., a flash in the surround, when properly timed, is effective in eliminating the response to change (a flash) at the center. The relatively small hyperpolarization elicited by the peripheral flash was effective in competing with the larger centrally elicited depolarization. This suggests that the two antagonistic components of the response may not simply add algebraically. The hyperpolarization might be an IPSP that "shunts" the membrane thereby reducing the effectiveness of subsequent excitatory inputs (Kuffler and Eyzaguirre, 1955) or only part of the lateral effect from the surround might be fed forward to the ganglion cell in the form of a hyperpolarizing transient; another signal might be fed back to bipolars to limit the response.

It was shown previously (Werblin, 1972) that an illuminated windmill pattern, when spinning in the far surround of the ganglion cell receptive field, was effective in antagonizing the central response. Fig. 4 C shows the response of an on-off ganglion cell elicited by a central flash in the presence of a stationary windmill, followed by the response to the same flash, now in the presence of the spinning windmill. The vanes of the windmill are truncated at 1 mm, so they lie in the same part of the peripheral receptive field as the flashing ring and spot in Fig. 4 A and 4 B but here the windmill background was steadily moved rather than flashed in the periphery. The ganglion cell membrane was steadily hyperpolarized in the presence of the spinning windmill, and the response to the central flash was reduced. Thus, the ganglion cell membrane was hyperpolarized either by spinning or flashing the surround, but the hyperpolarization in the presence of sustained spin was maintained. When ganglion cell activity was recorded extracellularly, the windmill always reduced the response to the central test flash. However, there was seldom any indication of spontaneous activity. The apparent spontaneous activity shown in Fig. 4 C might result from some depolarization of the ganglion cell membrane due to damage by the electrode.

Often the depolarization due to presumed electrode damage increased in magnitude during the recording and the spike frequency reached a maximum after which it ceased completely. The hyperpolarizing transients elicited by surround antagonism were usually augmented under these depolarized conditions, so they were more easily measured. In Fig. 4 D there was a dramatic hyperpolarization when the windmill began to spin, and the absolute potential level reached during the depolarizing transients was reduced in the presence of the spinning windmill.

These experiments indicate that spatiotemporal change in the surround, elicited either by the movement of the spinning windmill or the flashing peripheral spot or annulus, elicits hyperpolarizing activity in the ganglion cells, and that the response to test flashes at the center is reduced. About 25 on-off ganglion cells were studied in this way. In five the windmill elicited no hyperpolarization, but still suppressed the response to the center flash. The

on response was always more dramatically reduced than the off response. It was shown previously (Werblin, 1972) that bipolars at the center are not affected by the spin of the windmill. Similarly, Copenhagen (1972) has shown that bipolar activity is not affected by flashed peripheral spots that were of sufficient intensity to raise threshold in ganglion cells. Therefore, the lateral antagonistic effects elicited by flashes and movement are probably carried across the inner plexiform layer by neural processes. To test this notion we have studied the behavior of the amacrine cells under conditions used to elicit antagonistic activity in the ganglion cells.

Properties of the Amacrine Cell Response

The intensity-response relations for the amacrine cells resemble those for the on-off ganglion cells: they span less than one log unit along the intensity axis and tend to shift roughly parallel to each other as background luminance level is changed. The curves for one such amacrine cell at two different background levels are shown in Fig. 5. The lower ends of the curves can only be inferred, since the stimulator did not present test flashes between background and the first data point. The peak value of response was plotted in the curves. A series of recordings of the amacrine cell response to diffuse flashes of increasing intensity is shown in Fig. 6. These recordings show a significant feature of

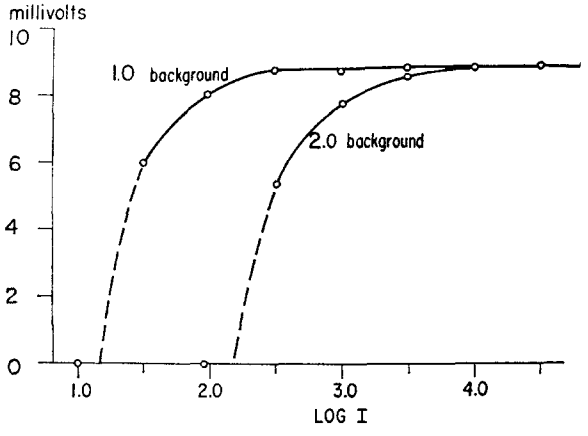

FIGURE 5

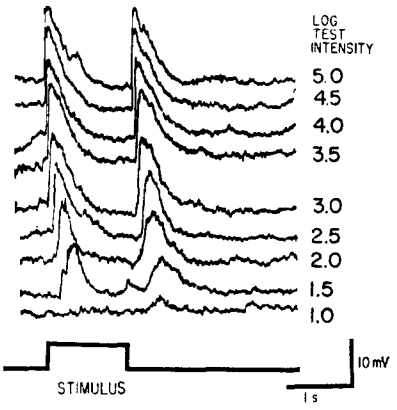

FIGURE 6

FIGURE 5. Intensity-response curves for an amacrine cell at two different backgrounds determined intracellularly. These curves tend to shift parallel to themselves as background is increased, and they span 90% of the response range in 1 log unit. Diffuse illumination was used as the test stimulus. The dashed curves are inferred in regions where no data points were taken.

FIGURE 6. Time-course of amacrine response to flashes of increasing intensity. The maximum peak response amplitude is reached for test flash about 1 log unit above threshold, although the latencies continue to decrease for increasing intensities. There is no clear sign of any competing hyperpolarizing antagonistic effect in these responses.

amacrine cell activity: in response to a diffuse flash of any intensity there is no apparent hyperpolarization with respect to the resting level. This waveform is quite different from that for the ganglion cells in which there was almost always some form of transient hyperpolarization, particularly when the stimulus extended well into the surround. Often, amacrine cells depolarize when damaged by the electrode and the response is reduced. The response can be reduced by passing depolarizing currents, but, unlike the ganglion cell recordings, the amacrine response shows no antagonistic component at any membrane potential level.

To test further for the possibility of a surround effect in the amacrine system we performed the same experiments outlined above that were useful for distinguishing the surround effect in ganglion cells. Fig. 7 A shows the response of an amacrine cell to a central and an annular peripheral flash. In both cases the stimulus elicited a stereotypical rapid depolarization, a single spike, and a gradual exponential-like decay of the response back to the base line. Although the response to the center and peripheral stimulus were of similar form, the response to the annulus occurred about 200 ms later than the response to the central test spot.

Fig. 7 B shows that when a peripheral spot was flashed 400 ms before the central test spot, it elicited an additional depolarizing response, but did not seem to antagonize the response to the central test spot. Fig. 7 C shows that when the windmill was spun it caused the amacrine cell at the center to be depolarized to a sustained level. Under these conditions the central test spot still elicited a depolarizing transient at on and off.

A comparison of ganglion cell and amacrine cell behavior under similar stimulus conditions as shown in Figs. 4 and 7 shows good correlation of some aspects of their electrical activity. Although test flashes at the center of the receptive fields always elicit depolarizing responses, flashing annuli or spinning vanes, 500 μm from the center of the receptive fields, hyperpolarized ganglion cells when they depolarized amacrine cells. This correlation suggests that amacrine cells, when driven by change in the periphery, carry a lateral antagonistic signal across the inner plexiform layer that causes the ganglion cell membrane to hyperpolarize.

Using the information derived from the above experiments we investigated the ways in which the antagonistic surround affected the intensity-response curves of the on-off ganglion cells by measuring response to a series of central stimuli of increasing intensity, in the presence or absence of change in the surround.

Effect of the Windmill on the Ganglion Cell Operating Curves

From the results of the previous experiments it appears that change in the surround of the receptive field of the on-off ganglion cells, either in the form

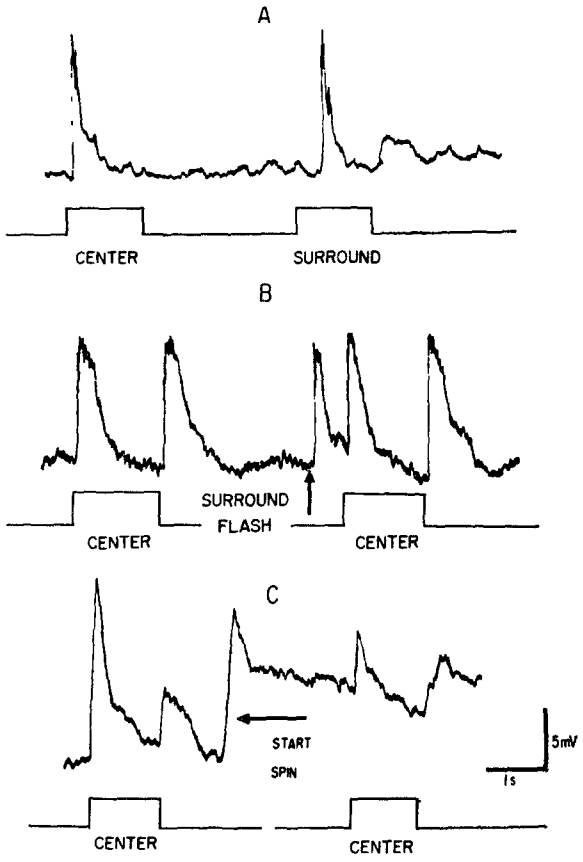

FIGURE 7. Amacrine response is modified by peripheral input. (A) Response to center (left) and peripheral (right) test flashes of the same intensity. Peripheral response appears about 200 ms later than the central response. (B) Effect of a peripheral flash of central response. The peripheral flash preceding the test by 400 ms, seems to add to the total response, so the response at on (right) is slightly larger than it was without the peripheral flash (left). (C) The windmill, when spinning, causes the amacrine cell membrane to *depolarize*.

of movement or flash, elicits activity in the transiently responding amacrine cells that in turn hyperpolarizes the ganglion cell membrane and limits the ganglion cell response to central stimuli. The graded ganglion cell activity in response to center test flashes was measured in the presence of both a spinning or a stopped windmill, and the results are shown by the graphs in Fig. 8. The time-course for each pair of recordings resembled the response curves in Fig. 4 C, and we measured the peak of the on transient not including the spike with respect to the instantaneous base line. The intensity of the windmill was set at a level such that when stationary it had no measurable antagonistic effect on the response of the ganglion cell.

In the presence of the spinning windmill, the ganglion cell intensity-response curve was shifted to the right, its apparent threshold was elevated, and the maximum elicitable response magnitude was reduced; even very bright stimuli were incapable of eliciting the level of depolarization possible with the windmill stopped. In other experiments, for example that shown in Fig. 11, we recorded extracellularly from the ganglion cell and monitored the total number of spikes elicited at on. The curves so generated resemble those shown here in Fig. 8. This correlation between the intracellular and extracellular response characteristics is useful: it indicates that the extracellular

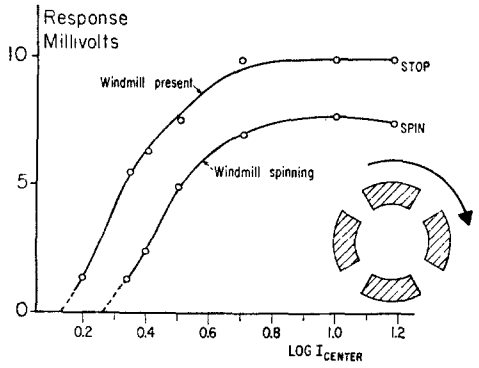

FIGURE 8. Effect of the spinning windmill on the graded on-off ganglion cell response. The intracellular response was elicited by the central flash with the windmill stationary, then spinning. The peak depolarization, not including the spikes, was measured. The windmill intensity was adjusted so that when stationary it had no effect on the ganglion cell graded response.

measurement of spikes is a good index membrane activity during excitation, and that the intracellular measurement was not distorted by cell damage.

Changes of the Ganglion Cell Operating Curves with Time

The lateral antagonism observed at the ganglion cell in the presence of the spinning windmill was sustained as long as the windmill was spinning (Fig. 4 C, D), whereas the effect of a flashed peripheral spot was only transient (Fig. 4 B). Therefore, the effect of the peripheral flash on the operating curves for the ganglion cell must be measured at the appropriate moment during the transient; about 250 ms following the flash according to Fig. 4 B. The time-course of threshold in the ganglion cell with respect to the onset of the peripheral flash is shown in Fig. 9. Threshold was determined by selecting a time interval between the presentation of peripheral background and the central test flashes, and then adjusting the intensity of the test flash on a number of trials until a *single spike* taken as threshold, was elicited. The experiment was repeated for a number of different intervals between background and test

flashes. The test flash used here was a 300-μm spot 490 ms in duration and the peripheral flash was an annulus with 1-mm inside diameter and 300 μm wide. These dimensions are not critical; Copenhagen (1972) has shown that the results are qualitatively similar even if the peripheral flash is a spot or a full field disk. The effect of the flashed surround was always markedly greater for the on response than for the off response. Also, the threshold was less dramatically elevated when measured with respect to the termination of the background than to its onset. This suggests that the on and off phases of response in the amacrine and ganglion cell systems are not functionally symmetrical.

The results shown in Fig. 9 indicate that threshold was most dramatically elevated at about 250 ms after the presentation of the background, and then decayed to a steady plateau over a period of about 1 s. In the following we compare the form of intensity-response curves before the background (A), at the plateau (B) and during the transient elevation (C).

Ganglion Cell Intensity-Response Curves During the Transient

The intensity-response curves for an on-off ganglion cell at different times with respect to the presentation of the background are shown in Fig. 10. Curve A, taken 520 ms before the presentation of the background serves as a reference curve before the transient threshold elevation. The presentation of the background elevated threshold most dramatically at 250 ms, but the effect was unmeasurable at that time, so we chose a time 620 ms after background to measure the intensity-response curve (C). Finally, curve B was taken at

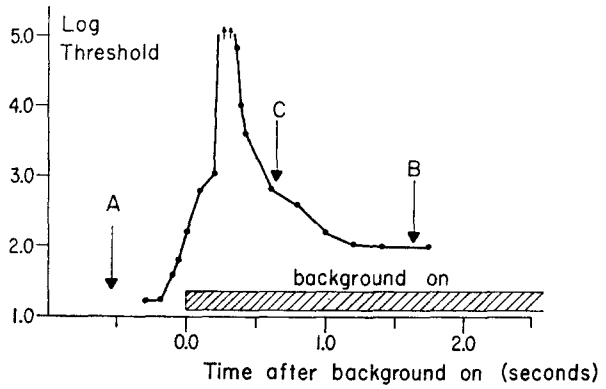

FIGURE 9. The time-course of threshold elevation in an on-off ganglion cell caused by the flash of a surround background. Abscissa shows the time of presentation of the central test spot relative to the background. Therefore 0.0 represents simultaneous flashes at center and surround. To the left of 0.0, the test flash is presented before the background. The test flash was 490 ms in duration. Maximum threshold elevation appears when the test spot is presented 250 ms after the background. Threshold then decayed to a plateau (B) after about 1 s.

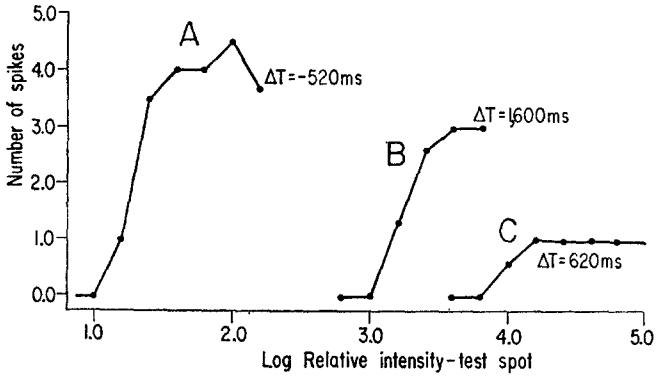

FIGURE 10. Effect of the peripheral flash on the operating curves of the on-off ganglion cell. (A) Curve at $T = -520$ ms for calibration, was generated before the presentation of the peripheral flash. (B) Curve at T = 1,600 ms taken during the sustained phase of threshold elevation. (C) Curve at T = 620 ms taken during the time of the threshold-elevating transient. Each point on the curve is the average total spikes of three trials.

1,600 ms at the plateau of the time-course of threshold elevation following the transient.

A comparison of the curves shows that the steady annular background acted to shift the response curve to the right along the long intensity axis (B), but that during the transient (C) after the flash of the surround annulus the curve was shifted further to the right and the response was compressed.

In a parallel set of experiments the spin of the windmill rather than the flash of the annulus was used to generate change in the surround as shown in Fig. 11. Again, as a reference the intensity-response curve (A) with no sur-

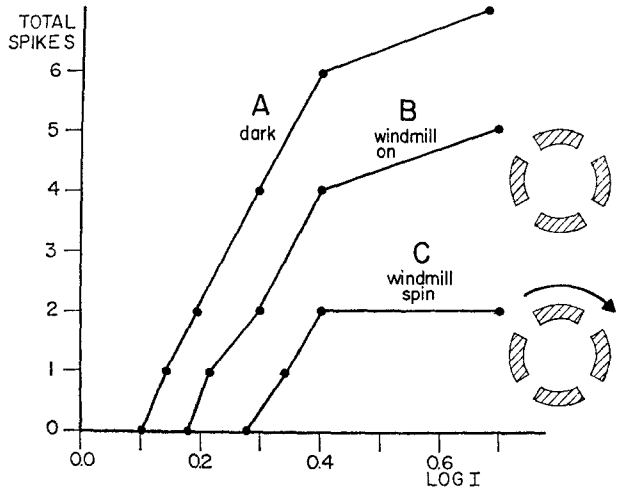

FIGURE 11. Effect of the windmill on the on-off ganglion cell response curves. Curve A plotted before the presentation of the windmill. Curve B plotted in the presence of the stationary windmill. Curve C plotted with the windmill spinning.

round was first determined. In the presence of the spinning windmill, (C), analogous to the transient threshold during the flash, the operating curve for the ganglion cell was shifted to the right and compressed. Finally, with the windmill present but not spinning (B) the operating curve was shifted to the right but not greatly compressed.

The pair of results given in Figs. 10 and 11 show a common effect of backgrounds in the periphery of the receptive field: steady backgrounds tend to shift the position of the intensity-response curves to the right (curves B); changing backgrounds, either in the form of flashing or moving patterns, shift the curves still further and compress the response (curves C). The effect of the windmill has always been less dramatic than the effect of the flashing annulus, probably because the flashing annular background elicits spikelike activity in the amacrine cell system, but the windmill elicits a depolarization of smaller magnitude.

Effects of Background on the On Type Ganglion Cells

A second major class of ganglion cell in mudpuppy, the on type ganglion cell, appears to have properties similar to those of the bipolars: these cells fire at a sustained rate in the presence of a central stimulus, and the sustained firing is tonically inhibited by a steady surround background. An example of this activity is given in Fig. 12.

It was shown previously that the spinning of the windmill had no apparent effect upon the activity of the bipolars (Werblin, 1972), and Copenhagen (1972) showed that a peripheral flashing background spot did not effect bipolar activity, although both backgrounds affected ganglion cell response. Therefore, if the on ganglion cells are driven directly by the bipolars with no interactions with the amacrine cell system, their activity should remain similarly unaffected by change in the surround. This notion is supported by the following experiments.

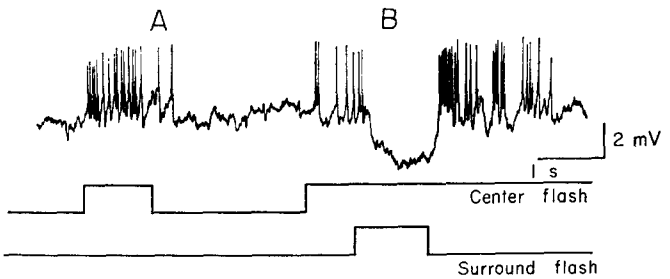

FIGURE 12. Time-course of center and surround effects in an on ganglion cell. The unit fired at a sustained level in the presence of the center stimulus (A), but this sustained activity was antagonized by the surround flash (B). The surround antagonism is accompanied by a hyperpolarization of the ganglion cell membrane. This experiment was performed under scotopic conditions.

The windmill used in these experiments had the same dimensions as that used previously in this report, namely, 1-mm inside diameter, but its intensity was increased so that, even when stationary, it tended to shift the intensity-response curves for the sustained on ganglion cell to the right. The results are shown in Fig. 13 for the same cell at two different background levels. The response curves at each background are indicated by circles; the curves in the presence of the stationary windmill are indicated by the squares. At each background the presence of the windmill acted to shift the curves parallel to themselves; (A to B; C to D) much the way an annular surround acted to shift the response curves for the bipolar cells shown in the previous paper (Werblin, 1974). However, the windmill when spinning, had no further affect upon these curves.

For comparison we showed in Fig. 8 that although a transient on-off ganglion cell was unaffected by a stationary windmill, it was affected by the spinning windmill; its operating curve was shifted to the right, compressed, and threshold was elevated. Thus the presence of the windmill alone but not the spin (Fig. 13), or the spin alone (Fig. 8) can affect the on or the on-off ganglion cells, respectively.

The cumulative effects of lateral interactions at both the outer and inner plexiform layers are shown in Fig. 11. The intensity-response curve for the on-off ganglion cells is aligned with a higher range of test flash intensities in the presence of the stopped windmill, (B) and then shifted even further to the right along the log intensity axis by the movement of the windmill (C). The shift from A to B is probably mediated at the outer plexiform layer, because a similar effect is seen in bipolar cells in the presence of a fixed surround background (Werblin, 1974). The shift from B to C, when the windmill is spinning, is probably mediated at the inner plexiform layer because this phenomenon is not observed in bipolar cells (Werblin, 1972). The sustained on-type ganglion cells are not affected by the spin of the windmill (Fig. 12) suggesting that they are unaffected by lateral interactions mediated by amacrine cells at the inner plexiform layer.

DISCUSSION

Surround Backgrounds, Center Stimuli, and Sensitivity Measurements.

The results reported here and in the previous paper (Werblin, 1974) show that the activity of the bipolar and ganglion cells can be modified by specific forms of background illumination presented at the surround of the receptive field for each cell type. Surround backgrounds affect the response for each cell to test stimuli presented at the center of the receptive field, and each effect can be interpreted in terms of a change in sensitivity at the center of the field. However, the configurations of the surround background and center test stimulus were used primarily for experimental convenience to isolate antago-

nistic response zones, so it does not necessarily follow that interpretations of sensitivity, based simply upon measurements of the antagonized center response, are generally acceptable criteria for evaluating retinal sensitivity. These criteria can be justified by the following arguments.

Retinal anatomy suggests that there is a direct synaptic pathway from receptors to bipolars to ganglion cells. In mudpuppy the center of the bipolar receptive field seems to be formed by the direct receptor to bipolar synapses and the sustained ganglion cells seem also to receive direct bipolar input (Werblin and Dowling, 1969). There is still some uncertainty about the synaptic input of the on-off ganglion cells but amacrine cells may be the major contributors (West and Dowling, 1973). An intervening synapse between bipolars and ganglion cells does not affect this argument. The point is that the "center," where sensitivity was measured, seems to correspond to an anatomically meaningful, limited set of synaptic connections between receptors and ganglion cells which exists regardless of the stimulus configuration. Furthermore, this center pathway has functional expression, in that the "center" response always seems to precede the surround antagonism at both the bipolar level (Werblin, 1974, Figs. 4 and 5) and the on-off ganglion cell level (Fig. 3), even when stimulated with a diffuse test flash. Therefore, regardless of stimulus configuration, the magnitude of the center response seems to be a meaningful index of retinal sensitivity. The center pathway, affected by lateral interactions at each plexiform layer, is illustrated in Fig. 14, where V_r, V_b, and V_g are the intracellularly measurable quantities in these experiments in receptors, bipolars, and ganglion cells.

Input-Output Relation at the Inner Plexiform Layer

It is possible to plot the relationship between peak bipolar potential and peak ganglion cell potential for a center test flash in the presence of two surround background conditions that affect lateral interactions at the inner plexiform layer. Fig. 15 A shows the ganglion cell response, versus log intensity, in the presence of both a spinning and stopped windmill, at scotopic levels taken from Fig. 8. In the previous paper (Werblin, 1974, Fig. 2) the response curve for a bipolar cell is plotted, also under scotopic background conditions. This response curve is reproduced in Fig. 15 B as a function of log test intensity. Then each pair of points from the bipolar and ganglion cell curves, corresponding to the response for a specific test intensity is plotted in Fig. 15 C. This curve shows the relation between peak ganglion cell and peak bipolar response for all test intensities, with a stopped and spinning windmill. When both responses are plotted with a common log intensity scale, the bipolar response is relatively linear with log I over the range of response for the ganglion cell. The ganglion cell response spans only a small portion of the bipolar response range, but it can be shifted by interactions at the inner plexi-

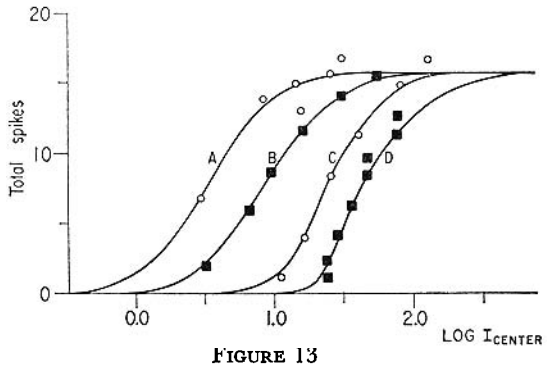

FIGURE 13

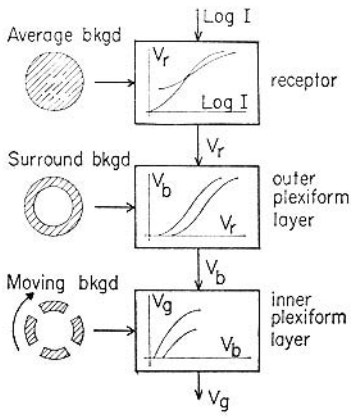

FIGURE 14

FIGURE 13. The intensity-response curves for an on-type ganglion cell. Curve A was generated in the dark, curve B generated in the presence of a stationary windmill. Curve C generated in the presence of a background. Curve D generated in the presence of the background plus the presence of the stationary windmill. In B and D, the subsequent spin of the windmill had no additional effect on the response, so no data for the spin effect alone is plotted. Test flash was 300-μm spot centered on the receptive field for the cell.

FIGURE 14. Scheme of concatenated events in the retina. The accessible signals in the retina are V_r, V_b, and V_g, the potentials in the receptors, bipolars, and ganglion cells, respectively. Each box contains the curves for the transfer functions at peak response from each accessible signal to the next, as derived in these papers. Test stimuli shown as log I elicit activity in the photoreceptors that is modified by average background conditions setting the level of adaptation. Receptor activity is related to bipolar activity through interactions at the outer plexiform layer modified by surround illumination. Finally, bipolar activity is related to ganglion cell activity modified by movement in the surround. The background conditions, imposed upon the surrounds of the receptive fields of the bipolar and ganglion cells, set the sensitivity at subsequent levels.

form layer to correspond with different regions of the bipolar response range as a function of flashing or spinning surround backgrounds. The steady background conditions used in the separate bipolar and ganglion cell experiments may not have corresponded exactly but minor shifts in either of the curves along the log intensity axis would not significantly alter the form of the input-output curves shown in Fig. 15 C.

The important point here is that threshold is apparently elevated in the ganglion cell because interactions at the inner plexiform layer raise the level of bipolar activity required to elicit a given level of response at the ganglion cell membrane. When ganglion cell activity is elicited, in the presence of spin, it appears to increase with a shallower slope with respect to bipolar activity. As shown below, both the decrease in slope and the shift in bipolar to ganglion cell input-output curve can act to reduce ganglion cell sensitivity.

Cumulative Effects of Sensitivity Control at Three Retinal Sites

A test flash that elicits a criterion response at the ganglion cell level elicits predictable levels of activity in the receptors and bipolars. From our results it is possible to derive the levels of peak activity elicited in each cell type corresponding to criterion threshold level at the retinal output, knowing the set of steady, surround, and transient background conditions. The curves in Fig. 16 summarize the relationships between activity in each cell type under specific background conditions. Curve B is the intensity-response curve for the rods, derived from the experiments in the accompanying paper (Normann and Werblin, 1974). Curves A are the input-output relations for the outer plexiform layer in the absence (1) and presence (2) of surround illumination as derived in the accompanying paper (Werblin, 1974). Curves C are the input-output curves for the inner plexiform layer taken from Fig. 15 showing ganglion cell activity as a function of bipolar activity for both a stopped (1) and a spinning (2) windmill.

If each of the input-output relations in A, B, and C is accurate, then it should be possible to derive the ganglion cell intensity-response curve from the concatenated input-output curves. For any log test intensity, a specific receptor potential V_r is determined by curve B. This receptor potential is related to a specific bipolar cell potential V_b under a given background condition in curves A. In this case the bipolar curve (1) was chosen. This bipolar potential is related to a specific ganglion cell potential, V_g for a given windmill condition. In C both the stopped (1) and the spinning (2) windmill curves were used. The relationship between curves A, B, and C determines the intersection of V_g with the initial log test intensity axis, and gives one pair of points on the ganglion cell intensity-response curves. When all points are determined in this way, the curves given by the two solid lines in D are generated. The points on the graph in D are taken from the experimental curves of Figure 8 in this paper, and the close fit between the experimental and derived curves serves as a rough verification for the form of the input-output functions in A, B, and C.

The input-output curves serve also to show how sensitivity can vary as a function of the relative slope and position for each set of curves, as controlled by specific background conditions. If we define sensitivity as before (Normann and Werblin, 1974, Werblin, 1974)

$$S \alpha \frac{dV}{d \log I} \cdot \frac{d \log I}{dI} = \frac{dV}{d \log I} \cdot \frac{k}{I_t},$$

then this can be expanded by the chain rule to include concatenated gain changes at each stage as

$$S \alpha \frac{dV_g}{dV_b} \cdot \frac{dV_b}{dV_r} \cdot \frac{dV_r}{d \log I} \cdot \frac{k}{I_t}.$$

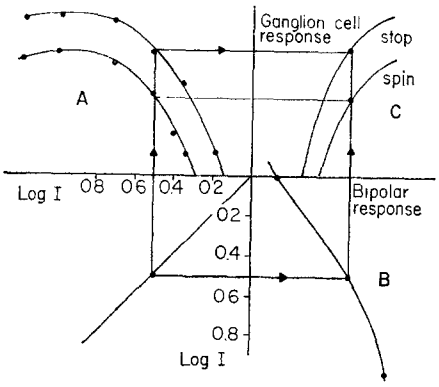

FIGURE 15

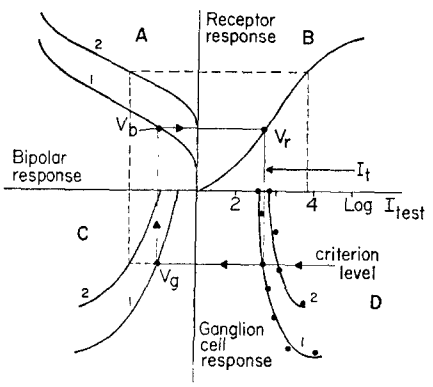

FIGURE 16

FIGURE 15. Derivation of the input-output curves for the inner plexiform layer. In curves C, the peak ganglion cell response, in the presence of a spinning and a stopped windmill, is correlated with the peak bipolar response to the same test intensities. Curves A, taken from Fig. 8, show the intensity-response function for a ganglion cell in the presence of a spinning and a stopped windmill, is correlated with the peak bipolar response to the same test intensities. Curves A, taken from Fig. 8, show the intensity-response function for a ganglion cell in the presence of a stopped and spinning windmill. Curve B shows the bipolar intensity response relation taken from Fig. 2 in Werblin (1974). For each test intensity, the peak ganglion cell response is plotted against the peak bipolar response in curves C. For example, a test flash intensity of 0.5 log units is projected to the ordinate and abscissa of curves C through the bipolar and ganglion cell intensity-response curves. In the presence of the windmill a greater bipolar response level is required to elicit ganglion cell activity, and the slope of the bipolar-to-ganglion cell curve is reduced.

FIGURE 16. Cumulative effects of sensitivity changes derived from the input-output curves. Curve B shows the intensity-response function for the rod as derived in (Normann and Werblin, 1974, Fig. 2). Curves A show the input-output relations for the outer plexiform layer, with no surround (1) and in the presence of a surround background (2), as derived in Werblin (1974). Curves C show the input-output relations for the inner plexiform layer in the presence of a stopped (1) and spinning (2) windmill. In D the intensity-response functions for the ganglion cell (solid lines), are derived by picking a no-surround condition (1) for the bipolars, and stopped windmill condition (1) for the ganglion cells, then plotting the ganglion cell potential elicited by different test flash intensities using the three transfer functions in A, B, and C. The points plotted in D are taken from the experimental data given in Fig. 8. The horizontal and vertical lines associate a test flash intensity of about 2.8 log units with a receptor potential level, V_r. Then V_r is associated, using the input-output relation in A, with a bipolar potential level V_b. Finally, V_b is associated with a peak ganglion cell response level, V_g using the input-output curves in C. V_g is associated with the 2.8-log unit test intensity to give the intensity-response relation for the ganglion cell in D.

Each of the terms in this expression has a specific representation in Fig. 16. For example, $dV_r/d \log I$ is given by the slope of curve B, dV_b/dV_r is given by the slope of curve A, dV_g/dV_b is given by the slope of curve C, and I_t is the test flash intensity for threshold at the criterion level. Sensitivity will be re-

duced if the slope of any of the input-output relations is reduced by background, or if the curves shift so that the value of I_t is increased.

The effects of each background condition upon the final level of ganglion cell threshold can be evaluated from the curves in Fig. 16. Assume first a steady background of 2.5 log units, but no surround or windmill. This defines the conditions for curves 1 in all cases. Starting at the criterion level, V_g in C, it is possible to determine the level of bipolar activity V_b from the curves in A. Knowing V_b it is possible to determine the concomitant V_r, and finally the required value of I_t the threshold test flash intensity, given here as about 2.75 log units.

Curves 2 in the figure are meant to represent the input-output curves at each retinal level in the presence of the background condition appropriate to elevate threshold by acting at that level, namely, a surround background in A, and a spinning windmill in C. Starting with the same criterion response in the ganglion cells, threshold is elevated by the cumulative effects of surround and windmill backgrounds as determined by following the dashed projection lines around the figure, to about 3.8 log units. The shifts in curves from conditions 1 to 2 are chosen here on the basis of the experiments in Fig. 8 and Werblin (1974) Fig. 8. They serve to show the relative effects of threshold elevation at each level.

The greatest component in threshold elevation is derived from the photoreceptors themselves. We showed previously that receptor response ranges can shift by as much as 7 log units in the presence of suitable steady backgrounds (Normann and Werblin, 1974). Although not shown in Fig. 16, the cone curves could be positioned any where over a broad range along the log I axis. However, the rod curves remain relatively fixed in position, so changes in the response curves of the more proximal cells can best be studied under the scotopic conditions used in these experiments. The bipolar response curves are shiftable within the range of receptor response, but this range corresponds to less than 3 log units (Werblin, 1974). Finally, the ganglion cell response curves are shiftable within the response range of the bipolars, but this range is only about 1.0 log unit (Fig. 10). Under some conditions, compression of the ganglion cell response curves alone can act to elevate threshold even more dramatically as shown in Fig. 9.

Two Plexiform Layers, Two Ganglion Cell Types

Our results suggest that two classes of ganglion cell activity are organized at different levels in the retina. The receptive field of the on-type ganglion cells, is formed at the outer plexiform layer, where center and surround antagonistic components respond with maintained activity to sustained illumination as in Fig. 12. The receptive field for the on-off ganglion cells is formed initially at the outer plexiform layer, then further modified through interactions at the inner plexiform layer. Therefore steady backgrounds and moving or changing

backgrounds are effective in modifying the response functions of the on-off units.

A similar dichotomy of response function was reported earlier by Enroth-Cugell and Robson (1966), who classified cat ganglion cells into the X-type (sustained) and the Y-type (transient), and showed similar differences in response properties. This dual classification has been pursued recently by Cleland et al. (1971, 1973), by Ikeda and Wright (1972), and by Saito et al. (1970). The results of these studies in the cat are consistent in many ways with the organization of two types of receptive field at the two plexiform layers, in mudpuppy. The X-type units respond tonically to small test spots at the center of the receptive field, and are unaffected by movement in the periphery. The Y-type units respond transiently to all stimulus configurations and are affected by movement in the receptive field periphery.

There is one important difference between the results of these studies and the results in mudpuppy. Sensitivity in the Y-type units can be *increased* by movement of a boundary in the far periphery of their receptive field. This so-called periphery effect was first reported by McIlwain (1964), and shown not to be a stray light artifact by Levick et al. (1965). Since the periphery effect requires movement of the peripheral boundary, and is not observed in the X-type units, it is probably mediated by the amacrine system, as suggested by Ikeda and Wright (1972). However, in terms of the data shown here, the effect cannot be due to direct input from the amacrine cell system to ganglion cells because that input appears to be inhibitory, at least in mudpuppy (Figs. 3 and 4) and *decreases* sensitivity (Figs. 10 and 11). The periphery effect could be related to a form of disinhibition at the inner plexiform layer, and that would require that the inhibitory lateral connections from amacrine cells be fed back, the only way to achieve disinhibition within a single level of neural processing (Ratliff, 1965).

Transient on-off ganglion cells have been found to respond to movement in a variety of animals including frog (Barlow, 1953; Maturana et al., 1960), pigeon (Maturana and Frenk, 1963) rabbit (Barlow and Levick, 1965), ground squirrel (Michael, 1968) and cat (Cleland et al., 1971). In each of these studies the magnitude of response to movement tends to increase as the size of the stimulus increases within the center of the receptive field. However, further increase in size of target leads to a diminution of response. Our results suggest that the outer regions of the test target, falling in the surround of the movement-sensitive receptive field, elicit windmill-like antagonistic activity in the receptive field surround tending to decrease the center response to movement.

SUMMARY

(*a*) The response characteristics of both the on and the on-off ganglion cells, to test stimuli presented at the center of the receptive field, could be modified

by a variety of surround background conditions. (*b*) The on ganglion cells respond with graded activity over about 2 log units of center test intensities. Surround illumination alters the range of log test intensities that elicits the graded response. (*c*) The on-off ganglion cells respond over somewhat less than 1 log unit of center test intensities. This narrow graded response range can be aligned with different ranges of center test flash intensities by the presence of surround illumination. In addition, movement in the surround further raised threshold, and compressed the magnitudes of response. (*d*) The results suggest that a receptive field that controls the response characteristics for the on and on-off ganglion cell is formed (through lateral interactions) at the outer plexiform layer. The response characteristics for the on-off ganglion cells are also altered by a second receptive field organization, formed through lateral interactions involving the change-sensitive amacrine cells at the inner plexiform layer.

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