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ANALYSIS OF THE HORIZONTAL CELL CONTRIBUTION TO THE RECEPTIVE FIELD SURROUND OF GANGLION CELLS IN THE RABBIT RETINA

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SUMMARY

1. The influence of horizontal cells on ganglion cells, the output neuron of the retina, was examined in an *in vitro* rabbit eyecup preparation. The extracellular spike activity of ganglion cells was monitored while pulsatile DC or sinusoidally modulated current was injected intracellularly into nearby horizontal cells. Interactions between the effects of light stimulation and horizontal cell current injections on ganglion cell responses were also examined.

2. Horizontal cells were found to contribute to the receptive field surround of ganglion cells. In particular, horizontal cells contributed to surround excitability and to surround antagonism of the centre light response.

3. Brisk, sluggish and direction-selective ganglion cells were all affected by current injections into horizontal cells. However, brisk ganglion cells responded to lower amplitude currents than did sluggish or direction-selective cells.

4. Horizontal cells with receptive fields that overlap those of ganglion cells were able to affect ganglion cell discharge. Moreover, the closer a horizontal cell was to the receptive field centre of a ganglion cell, the more effective were current injections in modulating ganglion cell discharge rate. The length constant of the horizontal cell contribution to the ganglion cell receptive field was approximately 200 μm. These results indicate that horizontal cells which are located within or outside of a ganglion cell's receptive field centre can influence that ganglion cell's activity.

5. The influence of horizontal cells on ganglion cell discharges was relatively weak at low temporal frequencies of sinusoidally modulated current.

6. Application of 2-amino-4-phosphonobutyrate (APB), a glutamate analogue, blocked the modulation of spike activity of on-centre ganglion cells that was induced by sinusoidally modulated current injected into nearby horizontal cells. The spike activity of off-centre ganglion cells was not blocked.

7. These findings suggest that horizontal cells contribute to the surround of ganglion cells and bipolar cells primarily through a feedback pathway onto cone photoreceptor cells.

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INTRODUCTION

In the vertebrate retina, horizontal cells, a type of second-order neuron with a large receptive field, make synaptic contacts in the outer plexiform layer and transmit information laterally from photoreceptor cells to other photoreceptors and to bipolar cells. In the retinae of poikilotherms, such as fish, turtles, and mudpuppies, horizontal cells contribute to the receptive field surround of bipolar and ganglion cells (Werblin & Dowling, 1969; Naka, 1971, 1977; Miller & Dacheux, 1976). Evidence in support of this scheme is provided by direct current injection experiments (Naka, 1971) and by ion-substitution experiments (Miller & Dacheux, 1976). For example, in the catfish and dogfish, current injections into horizontal cells can elicit discharges from nearby ganglion cells, hyperpolarizing current mimics the response of ganglion cells to light stimulation of their receptive field surrounds and depolarizing current mimics the response to light stimulation of the receptive field centre (Naka & Nye, 1971; Naka & Witkovsky, 1972). Moreover, hyperpolarizing current reduces the response of ganglion cells to stimulation of the receptive field centre, as does concomitant stimulation of the surround. Current injection experiments on pairs of nearby horizontal and bipolar cells in the turtle and fish have yielded similar findings (Marchiafava, 1978; Toyoda & Tonosaki, 1978).

Although bipolar cells in poikilotherm retinae possess clear surround responses (Werblin & Dowling, 1969; Kaneko, 1970; Miller & Dacheux, 1976), intracellular recordings of bipolar cells in the cat have generally not revealed surround responses (Nelson, Kolb, Robinson & Mariani, 1981; Nelson & Kolb, 1983). This finding, along with the absence of direct current injection experiments in the mammalian retina, has led to the suggestion that the surround of cat ganglion cells is mediated solely by inner retinal mechanisms and not by horizontal cells (Nelson *et al.* 1981; Nelson & Kolb, 1983; Kolb & Nelson, 1984).

Direct current injection experiments on nearby pairs of horizontal and ganglion cells in an *in vitro* rabbit preparation were therefore performed to study the role of mammalian horizontal cells in retinal function. In addition, the spatial and temporal characteristics of horizontal to ganglion cell transmission were quantified and the influence of horizontal cells on all of the types of ganglion cells, including brisk, sluggish and direction-selective cells (Cleland & Levick, 1974; Caldwell & Daw, 1978), was examined. Finally, the neuronal pathway from horizontal to ganglion cells was characterized by the bath application of a glutamate analogue, 2-amino-4-phosphonobutyrate (APB), during the current injection experiments.

METHODS

Preparation

Experiments were performed on superfused retinae obtained from pigmented rabbits approximately 2·0–4·5 kg in weight. A detailed description of the superfused rabbit eyecup preparation has been published previously (Miller, Zalutsky & Massey, 1986). Briefly, however, rabbits were deeply anaesthetized with urethane (1·5 g/kg, I.P.) and an eye was enucleated following additional local intraorbital injections of 2% Xylocaine. The eye was then hemisected and everted vitreal side up into a specially designed Teflon superfusion chamber that gently clamped the margins of the eyecup in place and allowed entry and drainage of the superfusate.

Rabbit superfusate was made according to the formula of Ames & Nesbett (1981), including organics and amino acids but excluding the horse serum. Absence of this latter component did not noticeably alter the health or longevity of the retina, as judged by physiological criteria such as the electroretinogram (ERG), intracellular recordings and ganglion cell extracellular single-unit activity. The superfusate flowed by gravity at approximately 3·5–4·0 ml/min, was maintained at about 36 °C with an in-line heater located near to the superfusion chamber, and was maintained at a pH of 7·4 by bubbling it with a mixture of 95% O_2 and 5% CO_2. A piece of tissue paper removed the superfusate from the superfusion chamber into a reservoir and maintained a superfusate depth of less than 1 mm above the retina. The health of the preparation was continuously monitored by an ERG. Experiments were discontinued when the C-wave of the ERG deteriorated.

Solutions and drug applications

DL-APB was purchased from Sigma Chemicals and was dissolved in the superfusate immediately prior to an experiment. The drug was oxygenated and maintained at a pH of 7·4 by bubbling it with a mixture of 95% O_2 and 5% CO_2, as was the control superfusate. The drug was applied to the retina by means of a switching valve located outside of the Faraday cage. Because the amount of dead space in the superfusion line is small, the effects of applications of DL-APB were observed within 25 s.

Electrophysiological recording and light stimulation

Simultaneous horizontal and ganglion cell recordings were obtained with intracellular micropipettes and tungsten-in-glass microelectrodes, respectively. Standard intracellular and extracellular recording techniques were utilized.

Intracellular pipettes were fashioned on a horizontal electrode puller (Campden Instruments) from omega-dot glass (o.d. 1·2 mm; i.d. 0·68 mm; Glass Company of America). Micropipettes with resistances between 100 and 200 MΩ when filled with 3 M-potassium acetate were able to impale horizontal cells and could be used to inject up to 15 nA of current. A current-to-voltage transducer, situated between the preparation and ground, was used to calibrate the amplitude of current injected intracellularly into horizontal cells.

The extracellular spike activity of single ganglion cells was monitored with glass-coated tungsten microelectrodes (Levick, 1972). Extracellular spike discharges were fed into a combined amplifier–window discriminator (Fintronics Corp. Model WDR-420), whose output was stored on a digital data-recorder (Instrutech Corp. Model VR-100) or recorded as spikes/s on a six-channel pen-recorder (Gould 260).

Light stimuli were provided by a triple-channel light bench early in this series of experiments or were generated on a video monitor, whose image was focused onto the retinal surface. Maximum luminance of the stimuli, as measured from a neutral test card (Kodak) of 90% reflectance that was situated at the retinal chamber in the stimulus path, was 2000 cd/m^2. In addition, a light background of 0·5 cd/m^2 was used throughout the course of all of these experiments to maintain the retina in the mesopic range. Light intensity was controlled by calibrated neutral density filters from log −6·0, to log 0·0 (unattenuated intensity). Stimuli used included spots of various diameters, annuli, full-field (diffuse) light and moving and flashing slits of various orientations.

Data analysis and cell identification

The lateral distance between the receptive field centres of simultaneously recorded horizontal and ganglion cells was determined using the responses to a slit of light slowly moving (1 deg/s) across the retina in two orthogonal directions. The time between the peak responses of both cell types was recorded for each direction of movement and then converted into distance. The distance between the cell types was then determined by the Pythagorean theorem.

These calculated values provide a physiological or functional measure of the lateral distance between horizontal and ganglion cell pairs. However, the values may not be anatomically exact because of a slight discrepancy between the physiologically determined receptive field centre of a cell and the exact anatomical location of its cell body. Moreover, the Pythagorean theorem holds for planar figures so that the spherical shape of the retina will introduce a slight discrepancy in the calculated values. However, it is likely that the calculated values of the lateral distance between

horizontal–ganglion cell pairs are quite close to the actual anatomical distance, probably with an error of no more than 5%.

The length constant, λ, of the horizontal cell contribution to ganglion cell receptive fields was determined using the above distance calculations and the exponential equation:

$$V_x = V_{\max} e^{-x/\lambda},$$

where V_x is the response of the ganglion cell when current is injected into a horizontal cell a distance x away and $V_{\max}$ is the response of the ganglion cell when current is injected into a coincident horizontal cell. The length constant is the horizontal cell-to-ganglion cell distance at which V_x equals $1/e(V_{\max})$ (Lamb, 1976).

Rabbit ganglion cell types were identified by previously established criteria (Oyster, 1968; Caldwell & Daw, 1978; Vaney, Levick & Thibos, 1981). Specifically, brisk and sluggish cells were distinguished by their different responses to quickly moving slits of light, the difference in the magnitude of their spontaneous activity, and the difference in the magnitude of their light-evoked activity. Direction-selective cells were identified by moving a spot across a cell's receptive field in various directions.

Recordings from cone-driven A- and B-type horizontal cell somata were distinguished from recordings of rod-driven B-type horizontal cell axon terminals on the basis of light response waveform (Bloomfield & Miller, 1982; Dacheux & Raviola, 1982; Raviola & Dacheux, 1983). Data from cone-driven A- and B-type horizontal cell somata are reported in this study; no simultaneous recordings of ganglion cells and rod-driven B-type axon terminals were obtained. Recordings of A-type horizontal cell somata probably cannot be distinguished from recordings of B-type somata on the basis of their light responses (Raviola & Dacheux, 1983). Because dye injections were not performed in this study, no attempt is made to distinguish between the two types of cone-driven rabbit horizontal cells. Because the soma of the A-type cell is the larger of the two, most of the horizontal cell recordings in this study were probably obtained from A-type cells. However, because data were obtained from seventy-one horizontal cells, it is likely that some of these recordings were from B-type horizontal cell somata. All cone-driven horizontal cells appeared to affect ganglion cells similarly.

RESULTS

Data were obtained from seventy-one horizontal–ganglion cell pairs. Each of the horizontal–ganglion cell pairs was located in the visual streak or within 5 mm on the inferior side of the visual streak. In addition, each of the horizontal cells was located within a lateral distance of 450 μm of the simultaneously recorded ganglion cell. Table 1 shows the number of pairings of horizontal cells with each type of ganglion cell, as well as the data base for each response property examined.

Horizontal cells contribute to ganglion cell surround excitability

Current injected into horizontal cells can alter the firing rate of rabbit ganglion cells. Moreover, hyperpolarizing current injected into horizontal cells produces changes in ganglion cell excitability which are equivalent to those produced by light stimulation of the ganglion cell receptive field surround (Mangel & Miller, 1987). This finding is illustrated in Fig. 1 for a horizontal cell–on-centre ganglion cell pair. Figure 1*A* depicts the light-evoked responses of a cone-dominated (A- or B-type) horizontal cell and of a simultaneously recorded on-centre brisk sustained ganglion cell to full-field light flashes of increasing intensity. The response or firing rate of the ganglion cell is displayed as spikes/s from a rate-meter record of the extracellular spike train. Figure 1*B* illustrates that pulsatile DC current (4 or 8 nA) injected into the horizontal cell modulated the firing rate of the ganglion cell. Specifically, hyperpolarizing current decreased, whereas depolarizing current increased, the

discharge rate of the ganglion cell, a relationship which was observed in every case in which an on-centre ganglion cell's firing rate was affected by horizontal cell polarizations (twenty of twenty cases), including both sustained and transient cells. In two of the cell pairs, no effect of the artificial horizontal cell polarization was observed. Moreover, increasing the magnitude of the current injection increased the amplitude of the change in the ganglion cell's firing rate (see also Figs 3 and 4).

TABLE 1. Data base for effects of horizontal cell polarizations on ganglion cell response properties

Ganglion cell type	Number of current injection pairings	Surround excitation	Intensity response	Surround antagonism	Spatial sensitivity	Temporal frequency sensitivity	Effect of APB
Brisk sustained							
On-centre	12	10	5	4	4	3	4
Off-centre	8	7	4	2	3	2	3
Brisk transient							
On-centre	10	10	3	2	2	3	2
Off-centre	6	5	2	1	4	2	1
Large field units	12	11	4	2	3	3	2
Sluggish sustained							
On-centre	4	3	2	1	3	2	2
Off-centre	1	1	—	—	1	1	—
Sluggish transient							
On-centre	3	1	1	—	1	1	—
Off-centre	5	3	1	—	3	2	—
Direction selective							
On-off	8	3	2	—	3	2	—
On	2	1	1	—	1	1	—

Hyperpolarizations of horizontal cells also affected off-centre brisk ganglion cells in a manner suggestive of a horizontal cell contribution to the surround excitability of ganglion cells. As shown in Fig. 2, hyperpolarizing current injections into horizontal cells increased the firing rate of off-centre ganglion cells and horizontal cell depolarizations decreased the firing rate. This phenomenon was observed in every case in which an off-centre ganglion cell's firing rate was affected by horizontal cell polarizations (twenty-three of twenty-three cases), including sustained and transient cells and large field units. In three of the cell pairs, no effect of the artificial horizontal cell polarizations was observed. Occasionally, when large amplitude pulsatile DC currents were injected, ganglion cell discharge was affected at the offset of current injection (see Fig. 2*B*).

These data suggest that horizontal cells contribute to the surround excitability of ganglion cells. That is, the firing rate of on-centre ganglion cells is increased by spot stimulation of the cell's receptive field centre and is decreased by annular stimulation of the cell's receptive field surround. Annular light stimulation also hyperpolarizes any nearby horizontal cells. Hyperpolarization of a nearby horizontal cell by intracellular current injection decreases the on-centre ganglion cell's firing rate, thus mimicking the effect of annular stimulation. Likewise, annular light stimulation or hyperpolarization of horizontal cells increases the firing rate of off-centre ganglion

cells. As mentioned above, these effects were observed in the vast majority of brisk ganglion cells studied, including both sustained and transient cells. Horizontal cell polarizations did not always affect nearby sluggish ganglion cells (see below) but when they did, the effects described above for brisk cells were also observed for

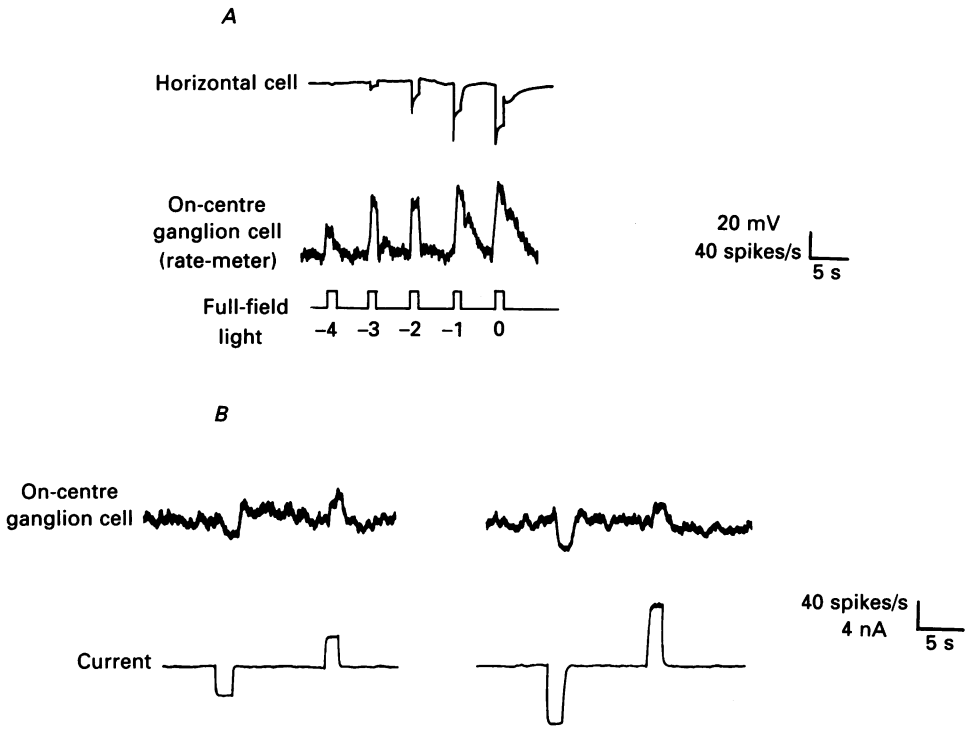

Fig. 1. Simultaneous intracellular horizontal cell and extracellular on-centre ganglion cell recordings and the effect of current injection into the horizontal cell on the spike activity of the ganglion cell in the rabbit retina. The horizontal cell was located about 100 μm laterally from this on-centre brisk sustained ganglion cell. The ganglion cell record is from a rate-meter and is shown as spikes/s. *A*, responses of both cells to a full-field light stimulus of increasing intensity. *B*, effect of DC current injected into the horizontal cell on the ganglion cell spike activity. Depolarizing current increased the firing rate of the ganglion cell and hyperpolarizing current decreased the firing rate.

sluggish cells. The only exception to these findings occurred when current was passed between horizontal cells and on–off direction-selective ganglion cells (see below). In this case, both horizontal cell depolarizations and hyperpolarizations increased the firing rate of the ganglion cells.

Because pulsatile DC current injections into horizontal cells generally altered ganglion cell activity in a transient manner (not shown but see Fig. 8), sinusoidal current, rather than DC current, injections were typically used. Sinusoidal current affected ganglion cell activity in a similar manner as that of pulsatile DC current (Fig. 3). For example, extrinsic hyperpolarizing current decreased the firing rate of on-centre ganglion cells (Fig. 3*A*, top two traces) and increased the firing rate of off-centre ganglion cells (Fig. 3*B*, top trace). The top trace in Fig. 3*A* depicts the actual

spiking of an on-centre ganglion cell to a current injection of 0·1 Hz. Rate-meter records of another on-centre ganglion cell and an off-centre cell are also shown in Fig. 3*A* and *B*, respectively. When extrinsic current of increasing amplitude is injected into nearby horizontal cells, the modulation depth of the extracellular activity of

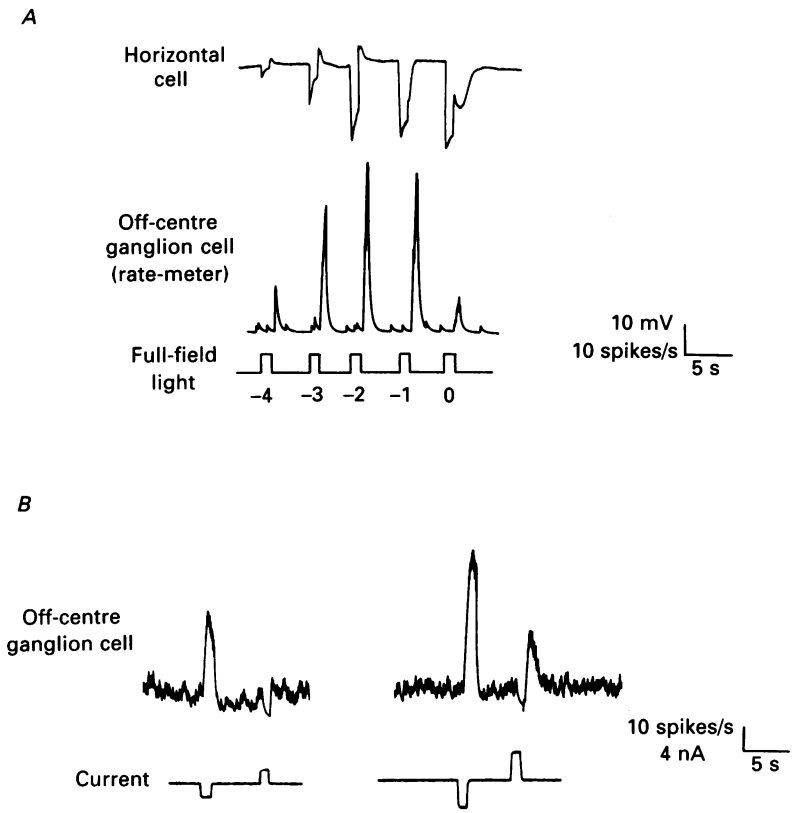

Fig. 2. Simultaneous intracellular horizontal cell and extracellular off-centre ganglion cell recordings and the effect of current injection into the horizontal cell on the spike activity of the ganglion cell in the rabbit retina. The horizontal cell was located about 150 μm laterally from this off-centre brisk sustained ganglion cell. Conventions are as in Fig. 1. *A*, responses of both cells to a full-field light stimulus of increasing intensity. *B*, effect of DC current injected into the horizontal cell on the spike activity of the ganglion cell. Depolarizing current decreased the firing rate of the ganglion cell and hyperpolarizing current increased the firing rate.

each of the ganglion cells is also increased (Fig. 3*A*, middle trace; Fig. 3*B*, top trace). However, following termination of the horizontal cell recording, sinusoidal current was injected through the pipette into the extracellular space near the site of the horizontal cell penetration. No effect of the extracellular current injection on the activity of either ganglion cell was observed (bottom traces of Fig. 3*A* and *B*). This latter control experiment indicates that modulation of ganglion cell excitability occurred only when current passed through the microelectrode into the horizontal cell.

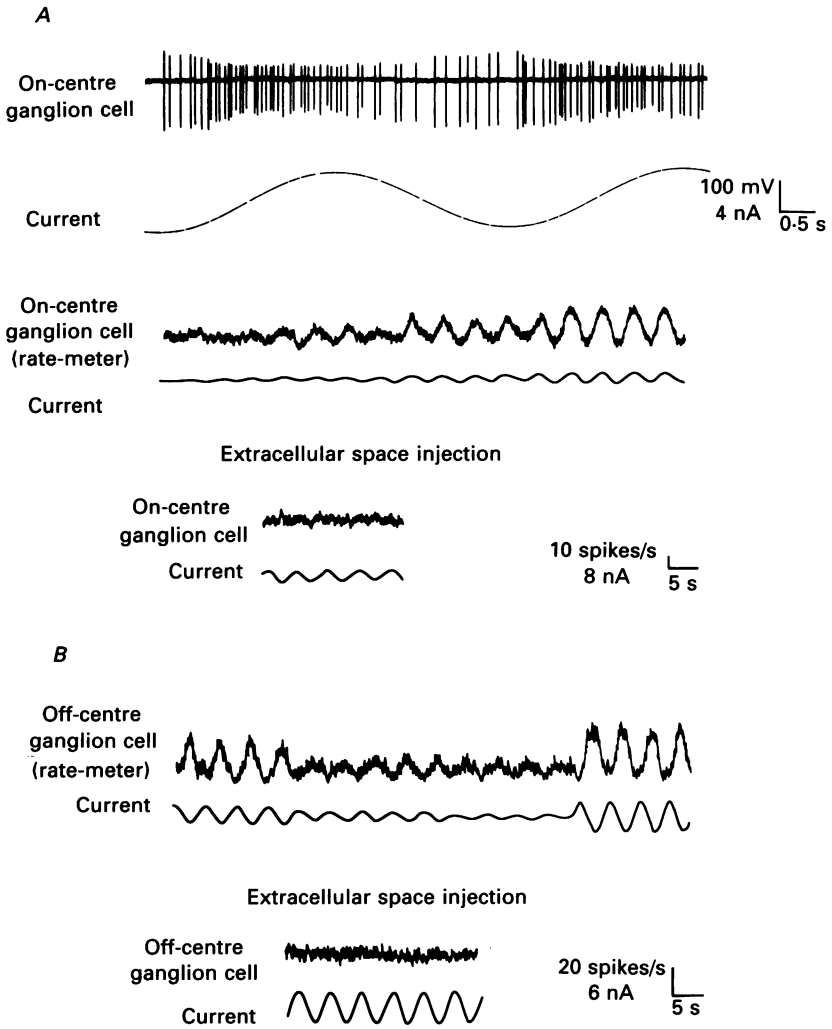

Fig. 3. Effect of sinusoidal current injections into horizontal cells on the spike activity of nearby ganglion cells. *A*, effects of horizontal cell current injections on an on-centre sluggish sustained ganglion cell (top portion) and on an on-centre brisk sustained ganglion cell (middle portion) are shown. The ganglion cell record in the top portion is the actual spike activity, whereas a rate-meter record in spikes/s that depicts the effects of variations in current amplitude is shown in the middle portion. The bottom portion illustrates a control experiment in which sinusoidal current was injected into the extracellular space near the site of the horizontal cell penetration. No effect of the control injection on the ganglion cell (same cell as in middle portion) was observed. Ganglion cell spike size decreases in the top trace with increased firing rate due to the limited frequency response of the pen-recorder. *B*, effect of variations in the amplitude of horizontal cell current injections on an off-centre large field unit in spikes/s (top portion) and lack of an effect of a control injection into the extracellular space near the site of the horizontal cell penetration on the same large field unit (bottom portion).

The relationship between the amplitude of the current injected into a horizontal cell and the amplitude of the resultant ganglion cell response is depicted in Fig. 4. Data from on-centre and off-centre ganglion cells are shown. Because the

effectiveness of artificial horizontal cell polarization on ganglion cell activity depends on the lateral distance between the horizontal cell and the ganglion cell (see Fig. 7), data were averaged from cell pairs that were separated by a relatively narrow range (between 50 and 250 μm apart). Moreover, the average lateral distance between the

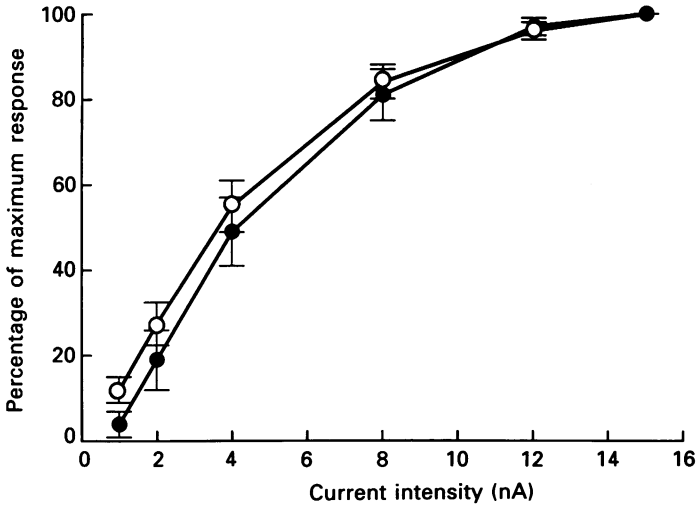

Fig. 4. Relationship between the amplitude of the current injected into horizontal cells and the amplitude of the resultant ganglion cell spike discharge. Spike discharge is expressed as a percentage of the maximum discharge. Averaged data from on-centre ganglion cells (●) and from off-centre ganglion cells (○) are shown. Because the effectiveness of current injections into horizontal cells on ganglion cell activity depends on the lateral distance between each horizontal–ganglion cell pair, data were averaged from cell pairs that were separated by a lateral distance of 50–250 μm (mean distance for on-centre cells was 160 μm; for off-centre cells, 135 μm). Bars indicate one standard error of the mean.

horizontal cells and the on-centre ganglion cells was 160 μm, whereas the average lateral distance between the horizontal cells and the off-centre ganglion cells was 135 μm. In addition, the spontaneous activity level of the ganglion cells used was high enough so that sinusoidal current injections of greatest magnitude (15 nA) modulated the firing rate of the ganglion cell without a complete block of activity.

If one assumes that the magnitudes of the horizontal cell polarizations were proportional to the amplitude of the injected current (see Sakuranaga & Naka, 1985), then Fig. 4 represents the relation between horizontal cell polarization and ganglion cell response. For small currents (less than 4 nA), the data plotted nearly on a straight line that intersected close to the origin. As current intensity increased, however, the relative effectiveness of the current in evoking ganglion cell spike activity progressively decreased, until saturation was reached at the highest intensities used. Although it is likely that this saturation of the current-induced ganglion cell responses is due to some feature of the chain of events from horizontal to ganglion cell, the possibility that it results from a decrease in horizontal cell membrane resistance brought about by excessive horizontal cell polarization cannot

be eliminated. However, the fact that large amplitude sinusoidal current injections modulated the spike activity of both on- and off-centre ganglion cells around a mean level argues against this latter possibility. Finally, the slightly greater effectiveness of horizontal cell current injections in affecting the activity of off-centre ganglion cells, compared to on-centre ganglion cells (see Fig. 4), may be attributed to the fact that the average lateral distance separating the horizontal cells from the off-centre ganglion cells was slightly less (135 μm) than that between the horizontal cells and the on-centre ganglion cells (160 μm) (see Fig. 7).

Horizontal cells contribute to surround antagonism of the ganglion cell receptive field

Classic experiments on mammalian retina demonstrated that light stimulation of a ganglion cell's receptive field surround affects ganglion cell responses in at least two ways (Kuffler, 1953; Enroth-Cugell & Lennie, 1975). First, surround stimulation increases an on-centre ganglion cell's discharge at stimulus offset and increases an off-centre ganglion cell's discharge at stimulus onset. This phenomenon can be called the 'excitatory surround' and can be observed with annular light stimulation alone. The data presented in Figs 1, 2 and 3, especially, support the view that horizontal cells contribute to the excitatory surround of ganglion cells. The second phenomenon can be called the 'antagonistic or suppressive surround' and denotes the case in which surround antagonism decreases discharge to a central spot of light at stimulus onset in on-centre ganglion cells or at stimulus offset in off-centre ganglion cells. The antagonistic surround can be observed when a central spot of light and an annulus are flashed simultaneously (see Figs 5 and 6).

Figures 5 and 6 demonstrate that rabbit horizontal cells also contribute to the antagonistic surround of ganglion cells. As shown in Fig. 5, increasing the intensity of a central spot of light progressively increased the size of an on-centre ganglion cell's response. However, if annular stimulation was present continuously or if hyperpolarizing current (10 nA) was injected into a nearby horizontal cell, the ganglion cell's responses to the same spot stimuli were dramatically decreased in size. Averaged data from this experiment (Fig. 6*A*) demonstrate that either annular stimulation of the ganglion cell's receptive field surround or hyperpolarizing current injected into a nearby horizontal cell can antagonize the centre response of the ganglion cell to spot stimuli. Moreover, Fig. 6*B* illustrates that when hyperpolarizing current of lower amplitude (4 nA) was injected into the same horizontal cell, the centre response of the ganglion cell was antagonized to a lesser extent.

Spatial–temporal characteristics of horizontal-to-ganglion cell transmission

In addition to a determination of the relationship between the intensity of horizontal cell current injection and ganglion cell response (see Fig. 4), the spatial and temporal characteristics of horizontal-to-ganglion cell transmission were also examined. To determine the spatial relationship between horizontal cells and current-affected ganglion cells, horizontal cell current threshold was determined as a function of the lateral distance between each horizontal–ganglion cell pair. The lateral distance between a simultaneously recorded horizontal cell and a ganglion cell was determined by moving a slit of light slowly across the retina at a constant

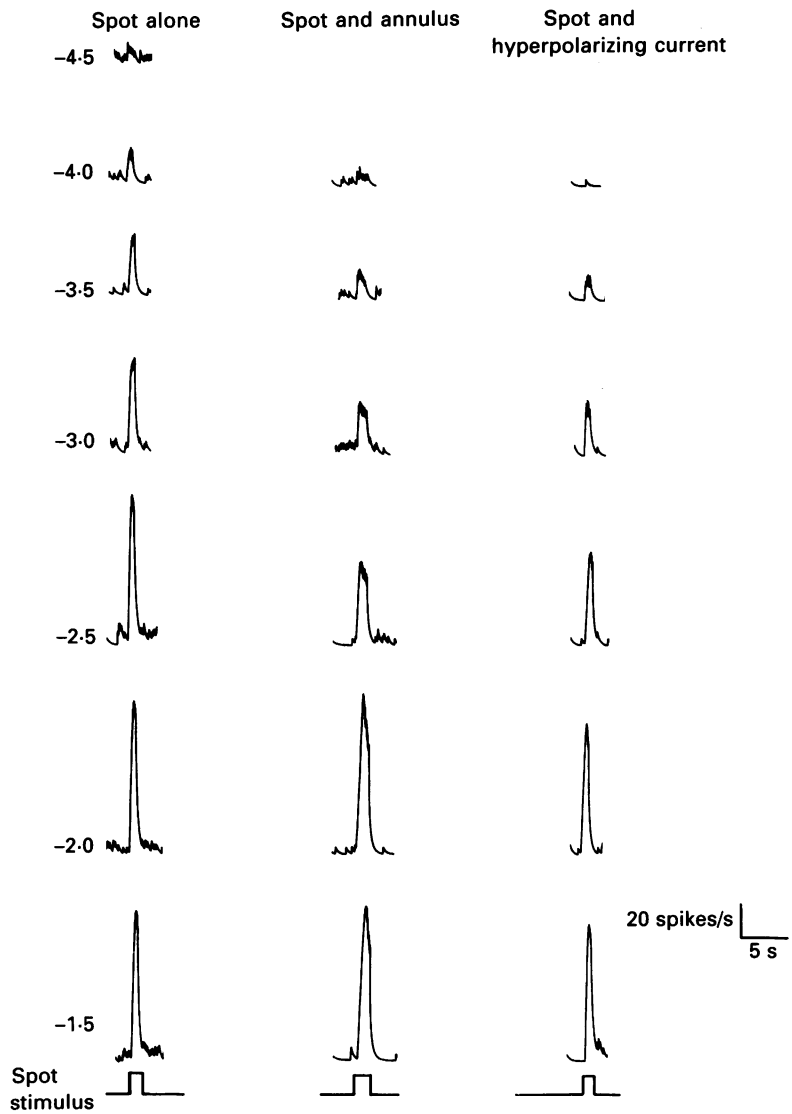

Fig. 5. Antagonism of the spot (receptive field centre) responses of an on-centre brisk sustained ganglion cell by light stimulation of the receptive field surround with an annulus or by hyperpolarizing current injection into a nearby horizontal cell. A rate-meter record in spikes/s depicts the response of the ganglion cell to spot stimuli of increasing intensity. A spot alone illuminated the receptive field centre of the ganglion cell or the spot was presented in conjunction with an annulus that continuously illuminated the receptive field surround of the ganglion cell at a constant intensity or the spot was presented in conjunction with the hyperpolarizing phase of a sinusoidally modulated current injection (10 nA at 0·1 Hz) into a horizontal cell located 225 μm laterally. The spot diameter was 400 μm. The inner diameter of the annulus was 750 μm and the outer diameter was 3 mm.

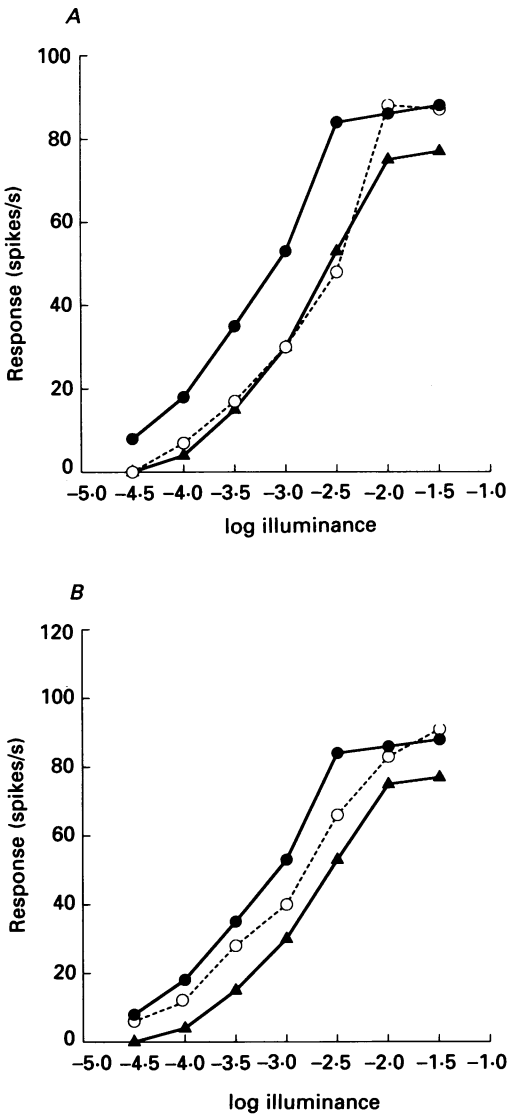

Fig. 6. Antagonism of the spot (receptive field centre) responses of an on-centre brisk sustained ganglion cell by light stimulation of the receptive field surround with an annulus or by hyperpolarizing current injections into a horizontal cell. Data were obtained as described in Fig. 5. Each data point represents the average response to five flashes of the spot stimulus. *A*, antagonistic effect of an annulus or of hyperpolarizing current (10 nA) injected into a horizontal cell on the spot response of the ganglion cell. ●—●, spot alone; ○---○, spot and annulus; ▲—▲, spot and hyperpolarizing current. *B*, magnitude of the antagonistic effect on the spot response of the ganglion cell is greater with larger amplitude (10 nA) current injections than with smaller amplitude (4 nA) current injections. ●—●, spot alone; ○---○, spot and 4 nA hyperpolarizing current; ▲—▲, spot and 10 nA hyperpolarizing current.

velocity (1·0 deg/s) in each of two orthogonal directions and by noting the timing of the resultant horizontal and ganglion cell responses (see Methods). For each horizontal–ganglion cell pair, horizontal cell current threshold was determined by

adjusting current amplitude (at 1 Hz) until the threshold of ganglion cell firing was reached, as determined by listening to spike activity on an audio-monitor. The reciprocal of this injected-current threshold value, namely current sensitivity, was then plotted as a function of the lateral distance between each of the horizontal–ganglion cell pairs (Fig. 7).

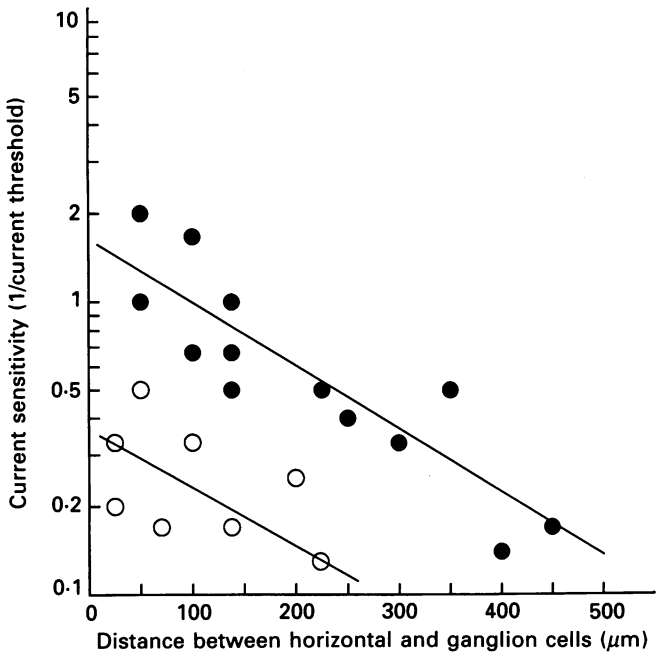

Fig. 7. Injected current sensitivity (reciprocal of current threshold) of brisk (●) and sluggish (○) ganglion cells as a function of the lateral distance between the horizontal–ganglion cell pairs. For each horizontal–ganglion cell pair, horizontal cell injected-current threshold was determined by adjusting current amplitude (at 1 Hz) until the threshold of ganglion cell firing was reached.

Current injected into horizontal cells was most effective in modulating ganglion cell activity when the horizontal–ganglion cell pairs were closest together, and was progressively less effective as the lateral distance between the cell pairs increased. This relationship occurred between horizontal–brisk ganglion cell pairs (correlation coefficient $r = -0{\cdot}88$) and between horizontal–sluggish ganglion cell pairs ($r = -0{\cdot}51$) but was clearest for the former cell pairs. Thus, the closer the centres of the receptive fields of a horizontal cell and a ganglion cell, then the stronger the effects of horizontal cell polarizations on the ganglion cell. In addition, at any lateral distance from a horizontal cell, current injections into that horizontal cell will be more effective in influencing the activity of brisk, compared to sluggish, ganglion cells.

The length constant of the horizontal cell contribution to the ganglion cell surround can be determined from the data shown in Fig. 7 (see Methods) and was approximately 200 μm. This suggests that the horizontal influence on ganglion cell activity may be observed even when the centres of a horizontal cell and a ganglion cell are 600 μm apart, that is, three length constants apart. In other words, a

horizontal cell that is three length constants away from a ganglion cell will affect the response of that ganglion cell by about 5% that of a horizontal cell that is coincident with the ganglion cell. On the other hand, the radius of rabbit ganglion cell receptive field centres rarely exceeds 500 μm (Vaney *et al.* 1981; Amthor, Takahashi & Oyster,

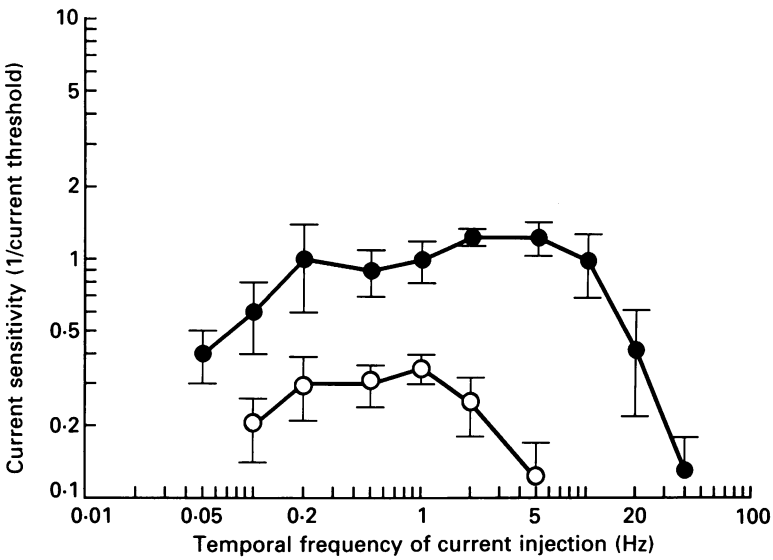

Fig. 8. Average current sensitivity (reciprocal of current threshold) of brisk (●) and sluggish (○) ganglion cells as a function of the rate of sinusoidal current modulation in Hz. At each temporal frequency of current modulation, the intensity of current injected into a horizontal cell was adjusted to a value at which the ganglion cell generated a threshold response. Bars represent one standard error of the mean contrast sensitivity at each temporal frequency.

1989). Thus, the horizontal cell contribution to ganglion cell surround excitability is not only most effective at the receptive field centre but also extends throughout the entire receptive field well beyond the receptive field centre.

To measure the temporal characteristics of horizontal-to-ganglion cell transmission, current amplitude was adjusted at each temporal frequency of sinusoidal current injection until the injected-current threshold was determined as noted above. Average current sensitivity (the reciprocal of current threshold) was then plotted as a function of temporal frequency (Fig. 8). As can be seen, the response of brisk ganglion cells is complexly related to the temporal frequency of horizontal cell current injections. In particular, horizontal cells influence brisk ganglion cell activity most effectively at an intermediate range of temporal frequencies. Interestingly, the influence of horizontal cells on brisk ganglion cell discharges is relatively weak at low temporal frequencies of current modulation (less than 0·2 Hz). As mentioned previously, DC current injections generally did not alter ganglion cell discharge in a noticeably sustained manner.

Compared to brisk ganglion cells, sluggish ganglion cell spike activity was difficult to modulate with horizontal cell current injections and could be modulated only at

relatively low temporal frequencies (Fig. 8). In fact, horizontal cell current injections could influence the extracellular activity of approximately only half of the sluggish ganglion cells monitored (eight of thirteen cases). Because the current threshold required to influence sluggish cells was relatively high, compared to brisk ganglion cells (see Fig. 7), it is possible that most or all sluggish ganglion cells could have been influenced, if the horizontal cell current injections had been of greater amplitude. An example of a horizontal cell current-induced modulation of an on-centre sluggish cell's firing rate is shown in Fig. 10*A*.

Effects of horizontal cell polarization on direction-selective ganglion cells

Current injections into rabbit horizontal cells could also influence the extracellular activity of direction-selective ganglion cells, in addition to brisk and sluggish cells. As shown in Fig. 9*A*, horizontal cell depolarizations and hyperpolarizations both increased the discharge rate of an on–off direction-selective ganglion cell, although relatively large amplitude currents were required to observe an effect on ganglion cell excitability. Figure 9*B* illustrates a control experiment in which identical sinusoidal current is injected through the micropipette into the extracellular space near the site of the horizontal cell recording. No effect of the current injection on the on–off cell of Fig. 9*A* is observed, indicating that ganglion cell excitability is affected only when current is passed into the nearby horizontal cell. Figure 9*C* also illustrates with a second horizontal-on–off direction-selective ganglion cell pair, using DC current injections, that both horizontal cell hyperpolarizations and depolarizations transiently increase the firing rate of the ganglion cell. On the other hand, horizontal cell depolarizations increase and horizontal cell hyperpolarizations decrease the firing rate of on-centre direction-selective ganglion cells (not shown), as occurs with all other types of on-centre cells.

Horizontal cells influence bipolar cells indirectly via cone photoreceptors

Although physiological studies of the retinae of cold-blooded vertebrates suggest that horizontal cells influence bipolar cells through a feedback pathway onto cone photoreceptors (Baylor, Fuortes & O'Bryan, 1971; Burkhardt, 1977), electron microscopy of mammalian retinae has indicated the possibility that horizontal cells synapse directly onto bipolar cells (Dowling, Brown & Major, 1966; Fisher & Boycott, 1974; but see Kolb, 1977). Thus, it has been suggested that horizontal cells influence bipolar cells directly in mammals, in contrast to cold-blooded vertebrates. To determine the pathway of horizontal cells to bipolar cells in the rabbit, 2-amino-4-phosphonobutyrate (APB), a synthetic glutamate analogue, was applied to the retina to study whether modulations of ganglion cell spike activity induced by current injections into horizontal cells are blocked by the drug. APB mimics the action of the endogenous photoreceptor transmitter on on-centre bipolar cells but has little effect on off-centre bipolar cells and horizontal cells (Slaughter & Miller, 1981; Bloomfield & Dowling, 1985). That is, APB blocks the light responses and decreases the membrane conductance of on-centre bipolar cells. Thus, if horizontal cells affect bipolar cells indirectly through cones, application of APB should block the horizontal cell-to-on-centre ganglion cell pathway at the bipolar cell level.

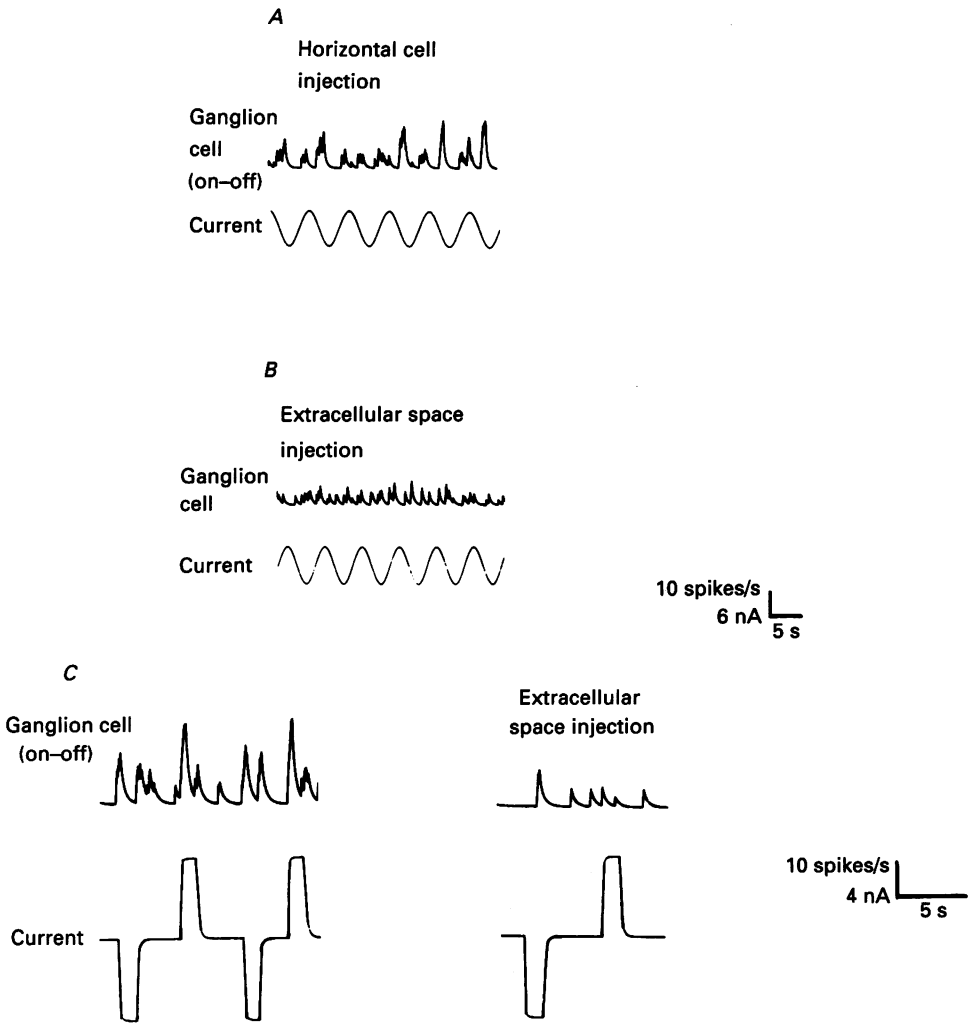

Fig. 9. Effect of current injected into horizontal cells on the spike discharge pattern of on–off direction-selective ganglion cells. Conventions are as in Fig. 3. *A*, sinusoidally modulated depolarizing or hyperpolarizing current injected into a horizontal cell increased ganglion cell discharge. *B*, control experiment in which sinusoidally modulated current was injected into the extracellular space near the site of the horizontal cell penetration. No effect of the control injection on the spike firing rate of the on–off direction-selective ganglion cell was observed. *C*, effect of DC current injected into a horizontal cell on discharge from a second on–off direction-selective ganglion cell (left side) and lack of an effect of a DC current injection into the extracellular space near the site of the horizontal cell penetration (right side). Both depolarizing or hyperpolarizing DC current injections into the horizontal cell transiently increased the discharge rate of the on–off ganglion cell.

Transmission from horizontal cells to off-centre ganglion cells should be unaffected. Alternatively, if horizontal cells contact bipolar cells directly, then APB application should not eliminate the effects of horizontal cell polarization on on-centre ganglion cells.

Figure 10 illustrates that application of APB (50 μM) blocked the effects of horizontal cell polarization on on-centre ganglion cells but did not diminish horizontal cell light responses (Fig. 10*A*). Furthermore, APB blocked the modulations of on-centre brisk and sluggish ganglion cell activity due to sinusoidal

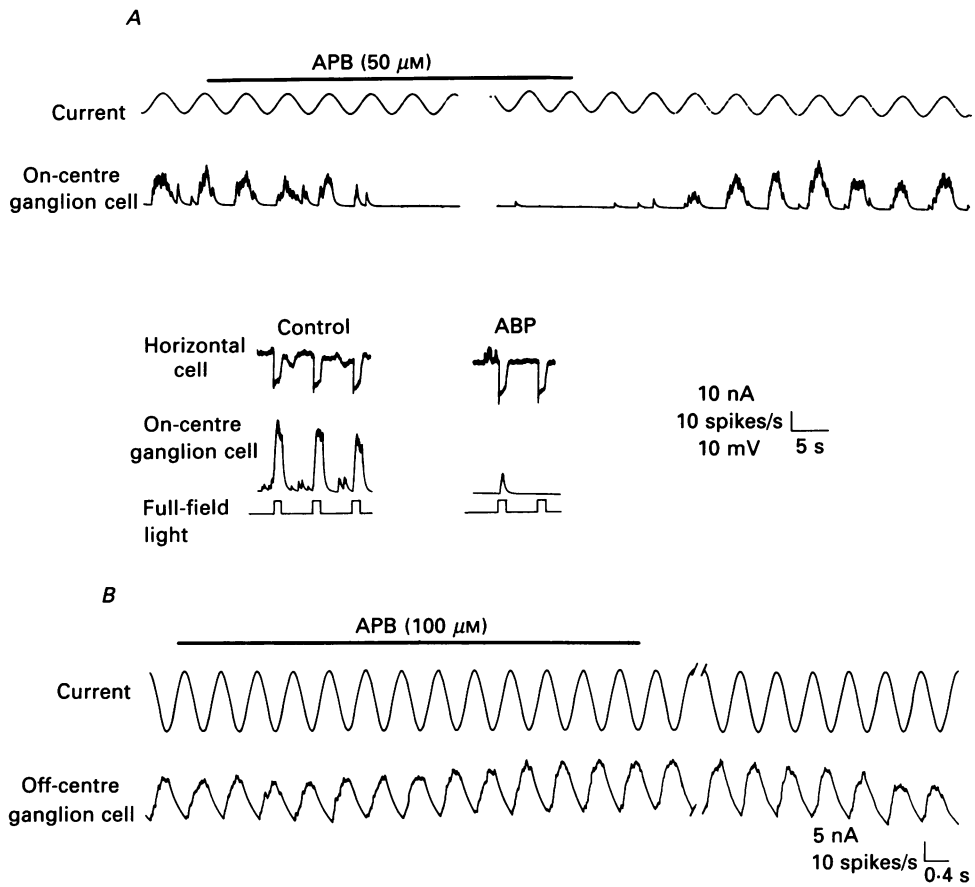

Fig. 10. Effect of APB application on the modulation of on-centre (*A*) and off-centre (*B*) ganglion cell spike activity induced by horizontal cell current injections. *A*, APB application (50 μM) blocked the modulation of spike activity of an on-centre sluggish sustained ganglion cell induced by sinusoidally modulated current injected into a horizontal cell. Before APB application both the horizontal and ganglion cell responded to full-field light stimulation but during APB application the response from the ganglion cell was almost completely abolished and the response from the horizontal cell was not attenuated (bottom part of *A*). *B*, APB application (100 μM) did not decrease the modulation of spike activity of an off-centre large field unit that was induced by sinusoidally modulated current injected into a horizontal cell. Rather, the spontaneous activity and the depth of modulation increased during APB application.

current injections into nearby horizontal cells in all cell pairings studied ($n = 8$). On the other hand, the modulation of spike activity of off-centre ganglion cells due to sinusoidal current injections into nearby horizontal cells is not blocked by APB (six

of six cases), as illustrated in Fig. 10*B*. Rather, the spontaneous activity and the depth of modulation increase during APB application (see Discussion below). Although APB eliminated the spontaneous activity of on-centre ganglion cells, small light-evoked responses remained in three out of eight cases (e.g. Fig. 10*A*). Because APB, at the concentrations used here, specifically blocks transmission from photoreceptors to on-centre bipolar cells in the rabbit (Bloomfield & Dowling, 1985), the findings reported here are consistent with the view that horizontal cell influence on on-centre (depolarizing) bipolar cells is primarily via an indirect feedback pathway onto cone photoreceptors.

DISCUSSION

The results of this study suggest that horizontal cells in the mammalian retina contribute to the receptive field surround of bipolar cells and brisk, sluggish and direction-selective ganglion cells primarily through a feedback pathway onto photoreceptor cells. Moreover, horizontal cells are most effective at modulating a ganglion cell's discharge rate when the distance between their somata is small and when the potential of the horizontal cell varies at an intermediate frequency range. These findings are discussed in more detail below.

Role of horizontal cells with respect to the ganglion cell receptive field surround

Studies on mammalian and non-mammalian vertebrate retinae have suggested that ganglion cell receptive field surrounds are constituted by contributions from both horizontal and amacrine cells (Naka & Nye, 1971; Enroth-Cugell & Lennie, 1975; Thibos & Werblin, 1978*a*, *b*; Caldwell, Daw & Wyatt, 1978; Mangel & Miller, 1987). Evidence presented in this study directly demonstrates that horizontal cells in the rabbit retina contribute to at least two aspects of the ganglion cell receptive field surround, namely, surround excitation and surround antagonism. Surround excitation, a phenomenon that can be observed with annular light stimulation alone, consists of an increase in an on-centre ganglion cell's discharge rate at stimulus offset, or of an increase in an off-centre ganglion cell's discharge rate at stimulus onset. The data presented in Figs 1 and 2, especially, directly indicate that horizontal cells contribute to the excitatory surround of ganglion cells. Surround antagonism, on the other hand, denotes the case in which stimulation of the surround decreases discharge to a central spot of light at stimulus onset in on-centre ganglion cells or at stimulus offset in off-centre ganglion cells. This phenomenon has been demonstrated for both cat (Enroth-Cugell & Lennie, 1975) and mudpuppy (Werblin & Copenhagen, 1974; Thibos & Werblin, 1978*a*) ganglion cells. The data shown in Figs 5 and 6 directly indicate that rabbit horizontal cells contribute to the antagonistic surround of ganglion cells. Interestingly, large amplitude focal injections into single horizontal cells were able to antagonize the central spot response of on-centre ganglion cells to the same extent as annular light stimulation of a relatively large retinal area (Fig. 6), a finding suggestive of horizontal cell coupling (Dacheux & Raviola, 1982).

Because horizontal cells in the fish retina (Naka & Nye, 1971; Naka & Witkovsky, 1972) also contribute to both ganglion cell surround excitation and antagonism, and

because rabbit and cat horizontal cells are morphologically very similar (Dowling *et al.* 1966; Fisher & Boycott, 1974), it is likely that horizontal cells in all vertebrate species play a similar role with respect to the receptive field organization of ganglion and bipolar cells. Moreover, the findings reported here suggest strongly that at least some bipolar cells in the mammalian retina possess receptive field surrounds, in agreement with a previous report of presumed rabbit bipolar cell recordings (Dacheux & Miller, 1981). As mentioned previously, other retinal elements, such as amacrine cells, may also contribute to ganglion cell surrounds (Thibos & Werblin, 1978*a*, *b*; Caldwell *et al.* 1978), so that ganglion cell surrounds, compared to bipolar cell surrounds, may be relatively more complex.

The spatial sensitivity profile of the horizontal cell contribution to the ganglion cell receptive field surround was also examined quantitatively in the present study. That is, the amplitude of current injected into each horizontal cell that was required to elicit a threshold change in the discharge rate of a nearby ganglion cell was determined as a function of the lateral distance between the horizontal cell and the ganglion cell. The reciprocal of these current threshold values, namely current sensitivity, when plotted as a function of the lateral distance between the horizontal and ganglion cells yielded a spatial sensitivity profile of the horizontal cell contribution to the ganglion cell surround. Figure 7 illustrates that the relationship can be approximated by a single-exponential function, such that the influence of horizontal cells on ganglion cell activity is greatest when the horizontal–ganglion cell pairs are closest together and is progressively less effective as the lateral distance between the cell pairs increases. These data support a model of receptive field organization, first proposed by Rodieck (1965) and Rodieck & Stone (1965). This model incorporates separate, symmetric and additive centre and surround mechanisms, such that the surround mechanism overlaps the centre mechanism in the middle of the receptive field and extends well beyond it spatially. The data reported here support this model and suggest, in addition, that the horizontal cell contribution to the receptive field surround of ganglion cells has its highest sensitivity in the middle of the receptive field and extends spatially beyond the receptive field centre. That is, the fact that the length constant of the horizontal cell contribution to the ganglion cell receptive field surround is about 200 μm (Fig. 7) suggests that horizontal cell influence on ganglion activity occurs even when the receptive field centres of a horizontal cell and a ganglion cell are 600 μm apart or three length constants apart. Thus, because the radius of rabbit ganglion cell receptive field centres rarely exceeds 500 μm (Vaney *et al.* 1981; Amthor *et al.* 1989), rabbit horizontal cells located outside of or within a ganglion cell's receptive field centre influence that ganglion cell's activity through an antagonistic surround mechanism.

In contrast to rabbit horizontal cells, the spatial sensitivity profile of the horizontal cell in the fish retina has been reported to decline with two exponential functions, a finding suggestive of electrical communication between horizontal cell body and axon terminal (Yagi, 1986). That the spatial sensitivity profile of the horizontal cell contribution to the ganglion cell surround in the rabbit declines with a single-exponential function, however, is not surprising, given that one type of cone-driven horizontal cell, the A-type, is axonless, and given that the cell body and axon terminal of the second type of cone-driven horizontal cell, the B-type, may not be

electrically connected (Nelson, Lutzow, Kolb & Gouras, 1975). In addition, the value of the length constant of the spatial sensitivity profile of the horizontal cell contribution to the surround in the rabbit, obtained here, is in approximate agreement with the value determined for the spatial sensitivity profile of cat horizontal cells (Nelson, 1977).

Neuronal pathways in the outer plexiform layer

The findings that APB, at the concentrations used here, specifically blocks transmission from photoreceptors to on-centre bipolar cells in the rabbit (Bloomfield & Dowling, 1985) and specifically blocks the effects of horizontal cell polarizations on on-centre ganglion cells (Fig. 10 here), are consistent with the view that horizontal cells influence on-centre (depolarizing) bipolar cells primarily via a feedback pathway onto cone photoreceptors. By logical extension, these findings suggest that horizontal cells influence off-centre (hyperpolarizing) bipolar cells primarily via cones as well. However, this above evidence, although suggestive, does not conclusively prove that rabbit horizontal cells release transmitter onto cones, and not directly onto bipolar cells. For example, if APB hyperpolarizes on-centre bipolar cells well below the threshold potential for the release of bipolar cell transmitter onto on-centre ganglion cells, then horizontal cell polarizations might not be able to depolarize on-centre bipolar cells sufficiently to cause ganglion cell spiking, even if the horizontal to bipolar cell connection is direct. On the other hand, two experimental findings argue against this supposition. First, as can be seen in Fig. 10*A*, the APB concentration used here did not block light-evoked ganglion cell spiking completely. Second, APB application increases the membrane resistance of on-centre bipolar cells (Slaughter & Miller, 1981), which would tend to augment the effects of horizontal cell polarizations on on-centre bipolar potentials and transmitter release. Be that as it may, until current injection/APB experiments are performed on horizontal–bipolar cell pairs, the exact neuronal pathway between horizontal and bipolar cells cannot be known with certainty.

Evidence from isolated fish bipolar cells and cone photoreceptors also supports the existence of a feedback pathway from horizontal cells to photoreceptors that would utilize GABA, the horizontal cell transmitter (Tachibana & Kaneko, 1984, 1987). Specifically, these workers found a high sensitivity to applied GABA at cone pedicles and at bipolar cell axon terminals but not at bipolar cell dendrites or somata, likely sites of horizontal cell transmitter action.

The polarity of the effects of horizontal cell polarization on on-centre and off-centre ganglion cells and on-centre and off-centre bipolar cells also suggests that horizontal cells feedback onto cones rather than feedforward onto bipolars. For example, horizontal cell depolarizations produce depolarizations in fish on-centre bipolar and ganglion cells (Naka & Nye, 1971; Toyoda & Tonosaki, 1978; Marchiafava, 1978) and in rabbit on-centre ganglion cells (Fig. 1). On the other hand, horizontal cell depolarizations produce hyperpolarizations in fish off-centre bipolar and ganglion cells (Naka & Nye, 1971; Toyoda & Tonosaki, 1978; Marchiafava, 1978) and in rabbit off-centre ganglion cells (Fig. 2). Therefore, if horizontal cells in fish and rabbit synapse directly onto bipolar cells, then the same horizontal cell transmitter will have a sign-conserving action on on-centre bipolar cells and a sign-inverting action on off-centre bipolar cells. Although possible (cf. Slaughter & Miller, 1981),

there is at present no evidence to support such a view. Conversely, if horizontal cells in fish and rabbit feedback onto cones with a sign-inverting action, then the above polarity findings of current injection experiments are easily explained. For example, a sign-inverting connection from horizontal cells to cones and a second sign-inverting connection from cones to on-centre bipolars yields a situation in which horizontal cell depolarizations result in on-centre bipolar depolarizations.

Structural correlates of feedback synapses from horizontal cells to photoreceptors, such as presynaptic vesicles or pre- and postsynaptic membrane densities, have not been observed (e.g. Schaeffer, Raviola & Heuser, 1982), except in catfish (Sakai & Naka, 1986) and human retina (Linberg & Fisher, 1988). It is possible, however, that transmission from horizontal cells to photoreceptors may occur by voltage-dependent carrier-mediated release, as described by Schwartz (1982, 1987; Yazulla & Kleinschmidt, 1983) or by transmitter diffusion through extracellular space (Piccolino, Witkovsky & Trimarchi, 1987). On the other hand, conventional synaptic contacts from horizontal cells onto bipolar cells have been observed (Marshak & Dowling, 1987; Linberg & Fisher, 1988), although they are rare in the mammalian retina (Kolb, 1977).

Because APB primarily acts on the on-centre bipolar cell (Slaughter & Miller, 1981; Bloomfield & Dowling, 1985), it is not surprising that application of APB did not block modulations of off-centre ganglion cell spike activity induced by sinusoidal current injections into nearby horizontal cells (Fig. 10*B*). Rather, the spontaneous activity and the depth of modulation of off-centre ganglion cell activity increased during APB application. These latter effects may be the result of non-synaptic effects of APB on off-centre bipolar cells (Slaughter, 1986), the result of suppression by APB of a tonic inhibitory input from on-centre bipolars onto off-centre ganglion cells or the result of a facilitation by APB of non-APB excitatory amino acid receptors (Arkin & Miller, 1987).

Horizontal cell influence on ganglion cell classes

In this present study, current injected into horizontal cells was found to affect the discharge rate of nearby brisk, sluggish and direction-selective ganglion cells, in agreement with previous findings that each of these ganglion cell classes possesses a centre–surround receptive field organization and that additional response properties, such as directional selectivity, originate from inner retinal mechanisms (Caldwell *et al.* 1978). Due to a low encounter rate, horizontal cell pairings with other ganglion cell classes, such as local edge detectors, were not achieved, so that, although likely, it is not certain that horizontal cell polarizations would affect these cell classes as well.

Interestingly, the amplitude of current injected into horizontal cells that was required to elicit a threshold change in the discharge rate of nearby ganglion cells was lower for brisk cells than for sluggish or direction-selective cells. Moreover, in some cases sluggish ganglion cells were not affected by large amplitude (10 nA) current injected into horizontal cells. It is probable that this difference in the effect of horizontal cell polarizations on sluggish and direction-selective ganglion cells is due to inner retinal mechanisms, such as GABA- or glycine-mediated inhibition (Caldwell *et al.* 1978) or to membrane phenomena in the ganglion cells themselves. In other words, the relatively low sensitivity of sluggish, compared to brisk, ganglion cells to

horizontal cell polarizations suggests that a substantial component of the receptive field surround of sluggish cells is generated in the inner plexiform layer, in addition to a contribution from horizontal cells.

The difference in the effects of horizontal cell polarizations on on–off direction-selective ganglion cells on the one hand, and on large field units on the other hand, is also interesting in its own right. Large field units are primarily off-centre ganglion cells with observable on-components in their receptive field centres and observable off-components in their receptive field surrounds (Barlow, Hill & Levick, 1964). However, despite the on–off nature of their light responses, large field units respond to horizontal cell polarizations as do other off-centre cells. On–off direction-selective cells, in contrast, respond with discharge increases to both horizontal cell depolarizations and hyperpolarizations (cf. Schwartz, 1973). One interpretation of this difference is that on–off direction-selective cells receive input from both on-centre and off-centre bipolar cells, whereas large field units receive input from off-centre bipolar cells and transient on–off amacrine cells. Presumably, the on-component of the large field unit response was not in evidence during the horizontal cell polarizations because the threshold to affect the on–off amacrine cells is relatively high, compared to the bipolar cells.

Conclusions

In summary, horizontal cells in the rabbit retina play a similar role with regard to the receptive field organization of ganglion and bipolar cells as they do in cold-blooded vertebrates. They contribute to the receptive field surround of ganglion and bipolar cells primarily via a feedback pathway onto cone photoreceptor cells. Moreover, horizontal cells in the rabbit retina appear to influence all classes of ganglion cells and their contribution to the surround of ganglion cells extends throughout the entire ganglion cell receptive field and is most effective at the receptive field centre.

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