

# Receptive Field Properties of Rod-driven Horizontal Cells in the Skate Retina

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**ABSTRACT** The large receptive fields of retinal horizontal cells result primarily from extensive intercellular coupling via gap (electrical) junctions; thus, the extent of the receptive field provides an index of the degree to which the cells are electrically coupled. For rod-driven horizontal cells in the dark-adapted skate retina, a space constant of  $1.18 \pm 0.15$  mm (SD) was obtained from measurements with a moving slit stimulus, and a comparable value ( $1.43 \pm 0.55$  mm) was obtained with variation in spot diameter. These values, and the extensive spread of a fluorescent dye (Lucifer Yellow) from the site of injection to neighboring cells, indicate that the horizontal cells of the all-rod retina of skate are well coupled electrically. Neither the receptive field properties nor the gap-junctional features of skate horizontal cells were influenced by the adaptive state of the retina: (a) the receptive field organization was unaffected by light adaptation, (b) similar dye coupling was seen in both dark- and light-adapted retinas, and (c) no significant differences were found in the gap-junctional particle densities measured in dark- and light-adapted retinas, i.e.,  $3,184 \pm 286/\mu\text{m}^2$  ( $n = 8$ ) and  $3,073 \pm 494/\mu\text{m}^2$  ( $n = 11$ ), respectively. Moreover, the receptive fields of skate horizontal cells were not altered by either dopamine, glycine, GABA, or the GABA<sub>A</sub> receptor antagonists bicuculline and picrotoxin. We conclude that the rod-driven horizontal cells of the skate retina are tightly coupled to one another, and that the coupling is not affected by photic and pharmacological conditions that are known to modulate intercellular coupling between cone-driven horizontal cells in other species.

## INTRODUCTION

The electrical synapse, mediated by unique intercellular transmembrane channels that constitute the so-called gap junction, is now recognized as one of the basic mechanisms for signal transmission in the nervous system of all animal species (Furshpan and Potter, 1959; Bennett, 1977; Llinas, 1985). The gap-junctional pores, formed by membrane-spanning protein complexes (connexons), permit the bidirectional passage of ions and small molecules, thus providing a pathway for both electrical and chemical communication between coupled cells. Of particular relevance

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to this study are the results of recent experiments indicating that the extent to which some cells are electrically coupled can be modulated by a variety of experimental conditions (Spray, Harris, and Bennett, 1981; Spray and Bennett, 1985; Neyton and Trautmann, 1986; Bennett, Barrio, Bargiello, Spray, Hertzberg, and Saez, 1991). In the vertebrate retina, good examples of this type of modulation are the light- and pharmacologically-induced changes in electrical coupling between cone-driven horizontal cells of teleosts, turtles, and amphibia (Teranishi, Negishi, and Kato, 1984; Piccolino, Witkovsky, and Trimarchi, 1987; Dowling, 1989; Dong and McReynolds, 1991).

It has been known for some time that the receptive fields of horizontal cells far exceed the extent of their dendritic processes (Tomita, 1965; Naka and Rushton, 1967; Kaneko, 1970, 1971; Lamb, 1976), a phenomenon that derives mainly from the extensive electrical coupling between horizontal cells of similar type (Yamada and Ishikawa, 1965; Kaneko, 1971; Kaneko and Stuart, 1980; Witkovsky, Owen, and Woodworth, 1983). However, there have been numerous reports based on *in situ* studies that the receptive field organization (and by implication, the extent of electrical coupling) of cone-driven horizontal cells can be dramatically altered by varying the adaptive state of the retina (Mangel and Dowling, 1985; Shigmatsu and Yamada, 1988; Baldrige and Ball, 1991; Dong and McReynolds, 1991; Umino, Lee, and Dowling, 1991). Moreover, there is abundant evidence that the photically induced changes in electrical coupling are mediated by dopamine (Shigmatsu and Yamada, 1988; Yang, Tornqvist, and Dowling, 1988; Dong and McReynolds, 1991), the neurotransmitter of at least one class of interplexiform cell in the retinas of teleosts, turtle, and amphibia (Dowling and Ehinger, 1975; Negishi, Teranishi, and Kato, 1985; Schutte and Witkovsky, 1991). Dopamine decouples the horizontal cell network, resulting in a decrease in the amplitude of the intracellularly recorded response to surround illumination; conversely, the reduced current spread from the impaled horizontal cell to its neighbors leads to an enhanced response to photic stimulation of the receptive field center (Negishi and Drujan, 1979; Piccolino et al., 1987; Dong and McReynolds, 1991). Further support for this view was obtained in studies demonstrating that the modulatory effects of both light and dopamine are often reflected in the degree of dye coupling between neighboring horizontal cells (Teranishi et al., 1984; Piccolino et al., 1987; Tornqvist, Yang, and Dowling, 1988; Baldrige and Ball, 1991; Umino et al., 1991), as well as in changes in the distribution of particle arrays that characterize the junctional membranes of electrically coupled cells seen in freeze-fracture micrographs (Wolburg and Kurz-Isler, 1985; Kurz-Isler and Wolburg, 1986, 1988; Baldrige, Ball, and Miller, 1987, 1989).

In contrast to the extensive body of information on electrical coupling and its modulation in cone-driven horizontal cells, very little is known about the gap-junctional properties of rod-driven horizontal cells. It is not clear, for example, whether the receptive field size of rod horizontal cells changes as a function of background illumination (but cf. Villa, Bedmar, and Baron, 1991), or whether neuroactive substances such as dopamine exert an influence on the intercellular coupling between these cells. Indeed, the interplexiform cells of some species appear to use neurotransmitters other than dopamine (Nakamura, McGuire, and Sterling, 1980; Rayborn, Sarthy, Lam, and Hollyfield, 1981; Kleinschmidt and Yazulla, 1984;

Marc and Liu, 1984; Brunken, Witkovsky, and Karten, 1986), and there is no a priori reason to expect that all interplexiform cells serve to modulate horizontal cell coupling. Thus, the main objectives of this study were to investigate the receptive field and gap-junctional properties of rod-driven horizontal cells using methods that have been applied successfully to studies on cone-driven cells.

A particularly attractive preparation for these purposes is the all-rod retina of the skate (Dowling and Ripps, 1970; Szamier and Ripps, 1983; Ripps and Dowling, 1991). In addition to having only rod photoreceptors, which ensures that any effects that may be observed are not induced by activation of the cone system, the horizontal cells of this elasmobranch have extremely large perikarya (Dowling and Ripps, 1973; Malchow, Qian, Ripps, and Dowling, 1990) that are suitable for obtaining long-term, stable, intracellular recordings even with repeated solution changes during superfusion (Ripps and Chappell, 1991). Another interesting feature of the skate retina relates to the identity of the putative neurotransmitter of its interplexiform cell. As noted above, not all interplexiform cells are dopaminergic, and present evidence suggests that at least one type of interplexiform cell in the skate retina uses GABA as its neurotransmitter (Brunken et al., 1986), although other retinal neurons (e.g., amacrine cells) may be dopaminergic (Bruun, Ehinger, and Sytsma, 1984).

In this study, the effects of background illumination and various neurotransmitters (dopamine, GABA, glycine) on the receptive fields of skate horizontal cells were studied in the eyecup preparation, and correlative experiments were performed using histological methods: intercellular coupling as revealed by the spread of a fluorescent dye, and gap-junctional morphology observed by freeze-fracture. The results of the various types of experimental approach were consistent in revealing profound differences in the properties of electrically coupled, rod-driven horizontal cells of skate, and those of cone-driven horizontal cells in other species.

## METHODS

### *Preparation*

Skates (*Raja erinacea* and *R. ocellata*) were obtained from the Marine Biological Laboratory (Woods Hole, MA) and maintained in tanks of circulating artificial sea water under a 12-h light-dark cycle. A small rectangular strip of eyecup ( $\sim 1.5 \times 1.0$  cm), excised under dim red light from the tapetal region of a dark-adapted ( $> 2$  h) animal, was mounted on the stage of a water-cooled chamber ( $14^{\circ}\text{C}$ ) with its scleral surface resting on a scintered silver plate that served as the reference electrode for both intra- and extracellular recordings. The preparation was either kept under a stream of moist oxygen (light-adaptation experiments) or superfused with an oxygenated elasmobranch Ringer solution (pharmacological experiments) that contained (mM) 250 NaCl, 6 KCl, 20  $\text{NaHCO}_3$ , 1  $\text{MgCl}_2$ , 4  $\text{CaCl}_2$ , 0.2  $\text{NaH}_2\text{PO}_4$ , 360 urea, 10 glucose, and 5 HEPES, pH 7.6; drugs were added to the solution in various concentrations without substitution. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Superfusates were delivered, at a rate of 1–2 ml/min, through capillary tubing to the retinal surface of the preparation and evacuated by the siphoning action of Kimwipe strips placed on the edge of the eyecup. Solutions were saturated with pure  $\text{O}_2$  before each experiment, and were under  $\text{O}_2$  pressure throughout the experiment. In switching between superfusates, there was a delay time of 4–7 min for complete solution exchange in the recording chamber.

### *Electrical Recordings*

Glass micropipettes filled with 3 M KCl, and having resistances (in Ringer) ranging from 50 to 100 M $\Omega$  were used to record the light-evoked intracellular responses of horizontal cells (S-potentials). Signals were led through the salt bridge to a chlorided silver wire connected to the input stage of a negative-capacitance amplifier (Axoprobe-1; Axon Instruments, Inc., Burlingame, CA), recorded on an ink-writing oscillograph (model 2200S; Gould Inc., Cleveland, OH), and stored and analyzed using the pCLAMP program (version 5.5; Axon Instruments, Inc.) run on an IBM-AT computer. Horizontal cells were usually impaled at a depth of  $\sim 100$   $\mu\text{m}$  from the surface of the retina, but no attempt was made to identify (e.g., by dye-labeling after recording) the subtype of horizontal cell from which the recordings were obtained (Malchow et al., 1990). In any event, there were no detectable differences in the response characteristics of any of the cells from which recordings were obtained; their resting potentials were typically  $-25$  to  $-40$  mV, and their light responses were hyperpolarizing potentials with maximum amplitudes of 25–40 mV.

The condition of the preparation was monitored intermittently by recording the electroretinogram (ERG). A chlorided silver wire placed in the vitreous near the edge of the retina was led to the AC-coupled input of a differential amplifier (model DAM 50; World Precision Instruments, Sarasota, FL), the output of which was filtered through a low pass filter (100 Hz) and monitored on an oscilloscope. The preparation was discarded if there was any sign of deterioration in the transretinal ERG.

### *Optical System*

Stimulus fields were imaged on the preparation by a dual-beam photostimulator (Dowling and Ripps, 1971) consisting of two optically equivalent pathways that provided independent control of the exposure duration, spectral characteristics, area, and intensity of the two beams. The irradiances of the heat-filtered white light delivered by the pair of current-regulated halogen lamps were measured in the plane of the retina with a calibrated thermopile (Eppley Laboratory, Newport, RI) and microammeter. In experiments on the effects of light adaptation, the unattenuated retinal irradiances delivered by the test and adapting fields were 271.9 and 171.8  $\mu\text{W}/\text{cm}^2$ , respectively. The intensity values given in the text refer to the densities ( $D = \log T^{-1}$ , where  $T$  is transmissivity) of the calibrated neutral density filters used to attenuate the two fields; e.g.,  $I = -5$  corresponds to an incident irradiance of  $271.9 \times 10^{-5}$   $\mu\text{W}/\text{cm}^2$  for the test field and  $171.8 \times 10^{-5}$   $\mu\text{W}/\text{cm}^2$  for the adapting field. The dimensions of the stimulus fields were delimited by apertures in a plane conjugate with the retina. Depending on the experimental protocol, a variety of stimulus configurations were used: (a) a narrow slit (60  $\mu\text{m} \times 4.5$  mm) moved across the retina in 0.5-mm steps, (b) full-field illumination that covered the entire retina, (c) spot stimuli of varying diameters, and (d) concentric spot and annular fields that could be centered over the recording electrode by means of an X-Y micrometer drive.

### *Receptive Field Determinations*

For electrically coupled cells, the system can be treated as a two-dimensional network (Naka and Rushton, 1967; Lamb, 1976), in which the space constant provides an index of the electrotonic current spread. Assuming that the resistances of the cytoplasm and extracellular fluid are small enough to be ignored, the space constant ( $\lambda$ ) of the system is given by the expression:

$$\lambda^2 = R_m/R_j \quad (1)$$

where  $R_m$  is the membrane resistance ( $\Omega\text{-cm}^2$ ) and  $R_j$  is the gap-junctional resistance (ohms) of the cells. Under these conditions, the space constant represents the same distance at which the voltage response decays to  $1/e$  of its original value in a one-dimensional system.

Short of making direct measurements of the values of  $R_m$  and  $R_j$ , there are at least two ways in which to determine the space constant of the horizontal cell network. The first is to move a long, narrow slit of light of constant intensity across the retina and measure the response as a function of slit position. In this case, the voltage recorded from a cell located at a distance  $x$  from the center of an illuminated slit of width  $2a$  can be determined from the equation (cf. Lamb, 1976, Eq. 3):

$$\begin{aligned} V(x) &= V(o) [1 - \exp(-a/\lambda) \cosh(x/\lambda)] & |x| \leq a \\ &= V(o) \sinh(a/\lambda) \exp(-|x/\lambda|) & |x| \geq a \end{aligned} \quad (2)$$

where  $V(o)$  is the voltage recorded with the slit positioned over the cell. When the slit is very narrow,  $a$  approaches zero, and Eq. 2 reduces to:

$$V(x) = V(o) \exp(-|x/\lambda|) \quad (3)$$

An alternative method for determining the space constant is to use circular spots of varying diameter as stimuli. When a light spot of radius  $a$  is centered on the cell, the voltage  $V(\alpha)$  recorded at the cell will be (Lamb, 1976, Eq. 6):

$$V(\alpha) = V_f [1 - (\alpha/\lambda) K_1(\alpha/\lambda)] \quad (4)$$

where  $V_f$  is the voltage elicited by full-field illumination of the same intensity, and  $K_1$  is a modified Bessel function that describes electrotonic decay in a two-dimensional network (cf. Lankheet, Frens, and van de Grind, 1990).

#### *Pharmacology of Receptive Field Organization*

A convenient and sensitive means of testing for changes in receptive field organization is to compare the responses produced by a small spot of light with those produced by an annulus whose intensity is adjusted initially to elicit a response of equivalent amplitude; the horizontal cell response to a small, centered light spot is derived mainly from the direct input of photoreceptors, whereas the response to an annulus reflects primarily the input from electrically coupled neighboring horizontal cells. In the present experiments, a 0.5-mm-diam spot and an annulus with an i.d. of 0.8 mm and an o.d. of 5.0 mm were presented alternately at 30-s intervals. In the event of a drug-induced decrease in electrical coupling, the responses to the annuli (A) would be reduced in amplitude, whereas the responses to spot stimuli (S) would tend to increase (Negishi and Drujan, 1979); in other words, the A/S ratio would decrease.

#### *Dye Coupling*

Labeling of the cells with the fluorescent dye Lucifer Yellow (mol wt 457) was accomplished using the procedure described by Stewart (1978). The tip of the pipette was filled with 4% Lucifer Yellow in distilled water and the remainder was filled with 1 M LiCl; a small (0.1 nA) positive current was used to prevent dye leakage from the pipette. The dye was ejected by applying 10 nA negative current pulses intermittently (1 Hz) for 10–15 min. The retina was fixed overnight in 4% formaldehyde and 15% sucrose in 0.1 M phosphate buffer (pH 7.4); it was subsequently dehydrated in ethanol, cleared in xylene, and viewed with a Nikon inverted microscope equipped with fluorescence optics.

*Freeze-Fracture Images of Junctional Membranes*

The gap-junctional particle densities of skate horizontal cells from dark- and light-adapted retinas were determined from electron micrographs of freeze-fracture replicas. For dark-adapted preparations, the fish was placed in the dark for 2 h, after which the retina was removed under infrared illumination using an IR converter; light-adapted preparations were obtained under an ambient illumination of 2.16-ft lamberts. The isolated retinas were fixed for ~1 h in 2.5% glutaraldehyde prepared in 0.1 M sodium cacodylate buffer with 15% sucrose, and then cryoprotected by infiltration with 25% glycerol for 2 h. Pieces of retina (~1 × 2 mm) were mounted on standard gold stubs (Balzers, Hudson, NH) and frozen in supercooled liquid nitrogen (at -210°C). Samples were fractured and replicated on a BAF 400 T (Balzers) and the replicas were retrieved on Formvar-coated mesh copper grids and examined under the transmission electron microscope at 80 kV; the gap-junctional particle density was determined from enlarged micrographs.

## RESULTS

An example of the light-evoked responses from a skate horizontal cell to brief ( $t = 250$  ms) full-field stimuli of increasing intensity is shown in Fig. 1; the resting potential of the cell was about -35 mV, and the maximum (saturating) light response was a hyperpolarizing potential of ~40 mV. Fig. 1 *A* shows the waveform of the light responses, and Fig. 1 *B* gives the corresponding intensity-response relation. The solid line represents the best fit of the data to a Michaelis function (cf. Naka and Rushton, 1967; Dowling and Ripps, 1972) of the form:

$$V_1 = V_{\max} \frac{I}{I + \sigma} \quad (5)$$

where  $V_1$  is the response to a flash of intensity  $I$ ,  $V_{\max}$  (the maximum response of the cell) is 41 mV, and the value of  $\sigma$  (the intensity that evokes a half-maximal response) is approximately -5.65 log units, corresponding to a retinal irradiance of  $6.1 \times 10^{-4}$   $\mu\text{W}/\text{cm}^2$ .

*Receptive Field Properties in the Dark-adapted Retina*

The size of the horizontal cell receptive field was determined first under dark-adapted conditions with a narrow light slit of constant intensity as the test stimulus. The slit was moved across the retina in 0.5-mm increments, and the magnitude of the response was determined as a function of its location with respect to the center of the receptive field, i.e., the position at which the maximum response was elicited (Lamb, 1976). Recordings obtained from a horizontal cell using this paradigm are illustrated in Fig. 2 *A*, and a plot of response amplitude vs. slit position is shown in the lower half of the figure; the zero point on the scale of abscissas represents the intersection of the two exponential curves that best fit the data, and presumably indicates the location of the horizontal cell that gave rise to the responses. It is apparent that response amplitude decreased as the slit was moved to either side of the impaled cell. The data were well described by Eq. 3 with space constants of 1.26 and 1.39 mm (solid lines through data points); for 10 skate horizontal cells that responded in similar fashion, a space constant of  $1.18 \pm 0.15$  mm (mean  $\pm$  SD) was obtained. In some cells there were marked asymmetries between the two limbs of the response

function (data not shown). Although we suspect that this phenomenon arose when the impaled cell lay too close to the optic nerve head, the factors responsible for irregularities in the apparent receptive field organization of the horizontal cell were not explored further, and cells exhibiting such behavior were excluded when data were pooled for calculating receptive field parameters.

Another means by which to determine the size of the receptive field is to center a small spot of light over the cell, i.e., determine the location at which a maximal response is evoked, and then measure response amplitude as a function of spot diameter (Naka and Rushton, 1967; Lamb, 1976); the results of an experiment using

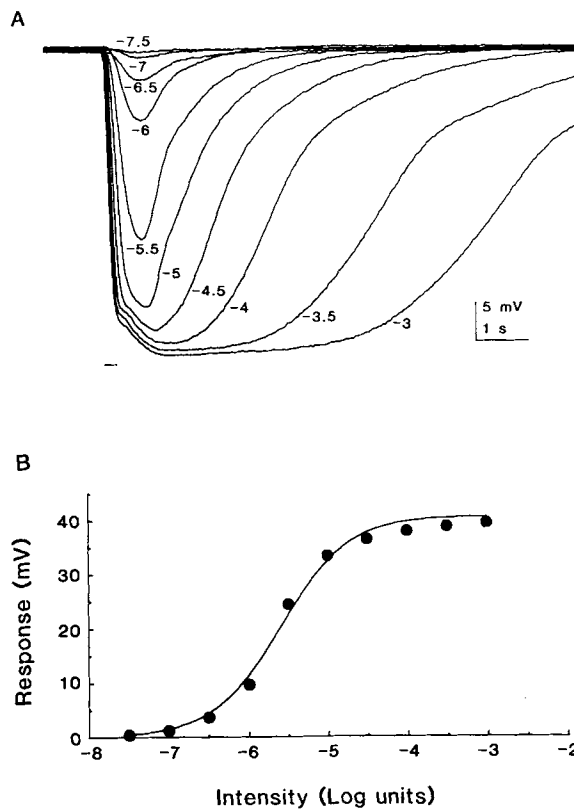


FIGURE 1. S-potentials recorded from a horizontal cell in the dark-adapted skate retina. (A) Light-evoked responses to full-field stimuli of increasing intensity (log  $I$  values next to each waveform); flash duration was 250 ms, indicated by the bar at the lower left of the traces. For this particular cell, the resting membrane potential was  $-35$  mV, and the maximum hyperpolarizing response to photic stimulation was  $-40$  mV. (B) The intensity-response function for the same cell. The filled circles represent peak responses from the traces shown in A, and the continuous curve is a least-squares fit to Eq. 5.

this approach are illustrated in Fig. 2 B. Responses to light spots varying in diameter from 0.143 to 8.5 mm show clearly the dependence of response amplitude on spot size, and the lower portion of the figure depicts the data graphically. The data points were fit well by Eq. 4, the expected relation between response amplitude and spot diameter for horizontal cells electrically coupled in a two-dimensional network. The results obtained for six horizontal cells tested with this protocol gave an average space constant of  $1.43 \pm 0.55$  mm (mean  $\pm$  SD).

*Effects of Background Illumination*

After characterizing the size of the receptive field of a cell under dark-adapted conditions, the measurements were repeated in the presence of steady background fields that illuminated the entire retina; sufficient time was allowed for the cell to adapt to the prevailing illuminance (cf. Dowling and Ripps, 1971). Fig. 3 shows the

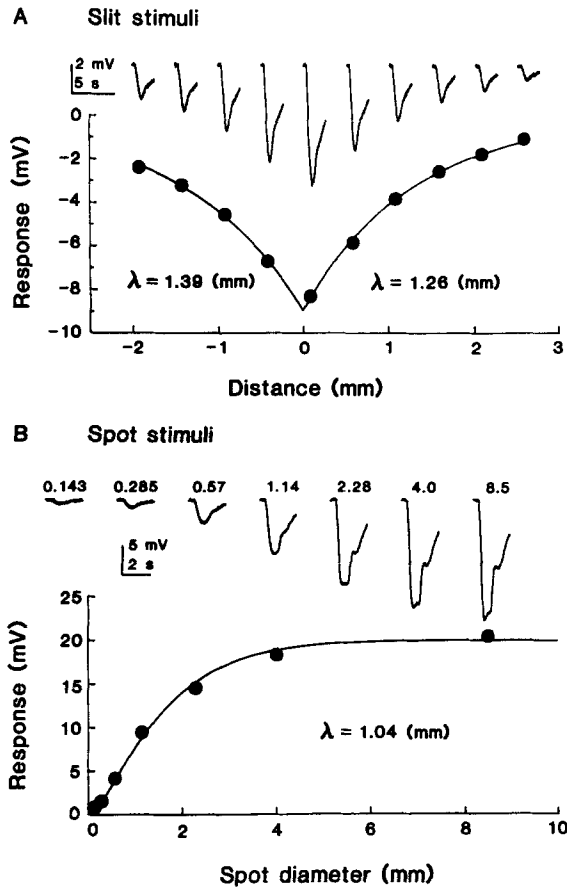


FIGURE 2. Receptive field data from dark-adapted skate horizontal cells obtained with slit (A) and spot (B) stimuli. (A) Recordings in the upper half of the figure show potentials elicited in response to 100-ms stimuli presented as a narrow ( $60 \mu\text{m} \times 4.5 \text{ mm}$ ) slit of white light of constant intensity ( $\log I = -3$ ), and moved in 0.5-mm steps across the retina; the traces have been adjusted for a slight baseline drift. The graph in the lower portion of the figure shows the peak response as a function of the position of the light slit. Lines connecting the points were obtained by determining for each limb the best fit to Eq. 3; the corresponding space constants are indicated on the graph. The junction point of the two limbs is assumed to be the point at which the light slit was located directly over the cell from which the recordings were obtained. (B) The upper half of the figure shows responses elicited by spot stimuli of fixed intensity ( $\log$

$I = -5.5$ ), but varying in diameter from 0.143 to 8.5 mm; the position of the traces has been adjusted for a slight baseline drift. The peak amplitudes of the responses from this cell are plotted in the lower half of the figure, where the continuous line represents the best fit of the data to Eq. 4 with a space constant of 1.04 mm.

results obtained from one horizontal cell using the moving slit paradigm to elicit S potentials across the receptive field. In this case, the responses were obtained first in the dark, then after 30-min exposure to a background field of  $-5 \text{ D}$ , and again after 30 min in the presence of a  $-3\text{-D}$  (100 times brighter) background. Note that both background fields evoked maximal (saturating) responses when first presented to the dark-adapted retina, but in each case the 30-min period of light adaptation was



sufficient for the cell to recover photic sensitivity and to reach a steady-state level of adaptation (Dowling and Ripps, 1971). Fitting the data to Eq. 3 gave average values (mean of the two branches) for the space constant of this particular cell of 1.32 mm in the dark, 1.29 mm in the presence of the dimmer background, and 1.36 mm with the brighter background. Similar results were obtained from eight additional cells. It is apparent, therefore, that background illumination did not affect the size of the receptive fields of rod-driven skate horizontal cells under these experimental conditions.

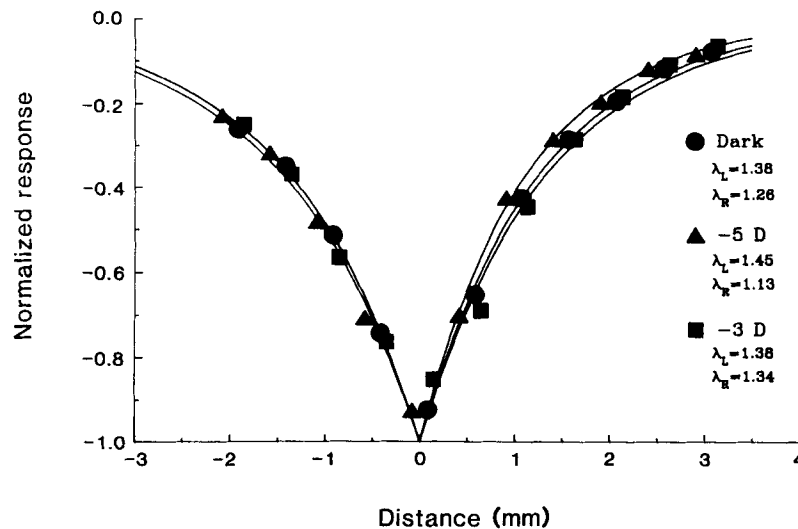


FIGURE 3. The effect of light adaptation on the receptive field of a skate horizontal cell. Receptive field measurements were obtained using the moving slit paradigm first in the dark-adapted retina (circles), then 30 min after exposure to a  $-5$ -D background (triangles), and finally after an additional 30-min exposure to a 100-fold higher background intensity ( $-3$  D; squares). The intensity of the light slit was chosen to elicit S-potentials of about the same peak amplitude under the different conditions of adaptation:  $-3$  D for the dark-adapted preparation,  $-2$  D with the dimmer background, and  $-1$  D when the brighter background was used. All traces are normalized to their response maxima, i.e.,  $-9.14$ ,  $-8.82$ , and  $-14.44$  mV, respectively. The curves are single exponentials, as described in Fig. 2 A.  $\lambda_L$  and  $\lambda_R$  refer to the calculated space constants for the left and right limbs, respectively, in each pair of curves. The differences among the space constants are within experimental error.

#### *Effects of Dopamine, GABA, and Glycine*

These drugs were of particular interest because of their association with interplexiform cells. As mentioned earlier, dopamine is a potent modulator of electrical coupling between cone-driven horizontal cells, whereas the skate retina is thought to possess a GABA-ergic interplexiform cell (Brunken et al., 1986). Glycine, on the other hand, is purported to be the neurotransmitter of some interplexiform cells in goldfish, toad, and *Xenopus* (Kleinschmidt and Yazulla, 1984; Marc and Liu, 1984), but no effects on the receptive field properties of horizontal cells have been reported.

The results presented in Fig. 4A show the potentials recorded from a horizontal cell in response to alternating flashes of spot and annular stimuli when the retina was superfused first with Ringer, then with 200  $\mu$ M dopamine, and again with the normal Ringer solution. The top traces are recordings of individual responses to spots and annuli obtained at the times marked by arrows on the bottom trace; the latter show on a slow time base, pen-writer recordings of the peak responses and resting membrane potential. The central line presents graphically the ratio of the peak responses evoked by annular stimuli to those obtained with spot stimuli as recorded throughout the course of the experiment. It is obvious that despite small drifts in resting potential and response amplitudes, the A/S ratio remained virtually unchanged; i.e., dopamine did not significantly alter the receptive field properties of the cell. Similar results were obtained from eight other cells.

Using the same protocol as in Fig. 4A, we found that the effects produced by 1 mM glycine (data not shown) and by GABA were similar to those obtained with dopamine. Even the application of as much as 10 mM GABA had no significant effect on the annulus/spot response ratio (Fig. 4B). Recordings obtained from 11 cells using GABA concentrations ranging from 500  $\mu$ M to 10 mM gave comparable results. Interestingly, 10 mM GABA did cause a depolarization of the horizontal cell membrane (Fig. 4B, bottom trace), consistent with previous findings indicating the presence of an electrogenic transport mechanism for GABA in skate horizontal cells (Malchow and Ripps, 1990).

#### *Effects of Bicuculline and Picrotoxin*

In goldfish and turtle retina, the GABA<sub>A</sub> antagonists picrotoxin and bicuculline, acting indirectly on a dopaminergic interplexiform cell, brings about a decrease in the electrical coupling between cone-driven horizontal cells (Negishi, Teranishi, and Kato, 1983; Piccolino et al., 1987). In skate, on the other hand, the results obtained with both compounds (Fig. 5) gave no indication of any effect on the receptive field organization of horizontal cells. Although there was a small (<10%), slow increase in the A/S ratio throughout the experimental run on one cell during exposure to 500  $\mu$ M bicuculline (Fig. 5A), results obtained in recordings from nine other horizontal cells showed that bicuculline, even at concentrations as high as 2 mM, had no effect on the membrane potential or the A/S ratio. Fig. 5B shows that picrotoxin was also without effect on the receptive field organization of the skate horizontal cell. In this case, superfusion of 500  $\mu$ M picrotoxin was entirely without effect on any of the response parameters (membrane potential, A/S ratio, response amplitudes) throughout the course of the experiment. Similar findings were obtained in seven other preparations.

#### *Freeze-Fracture Images of Gap-junctional Particles in Light- and Dark-adapted Retinas*

The morphological basis for electrical coupling is the gap junction between adjacent cell membranes. At these sites, the membranes of adjacent cells come into close apposition (a separation of only 2–4 nm), and the gap is bridged by membrane proteins that provide a channel connecting the interiors of the coupled cells. With the freeze-fracture technique, the gap-junctional proteins appear as a densely packed

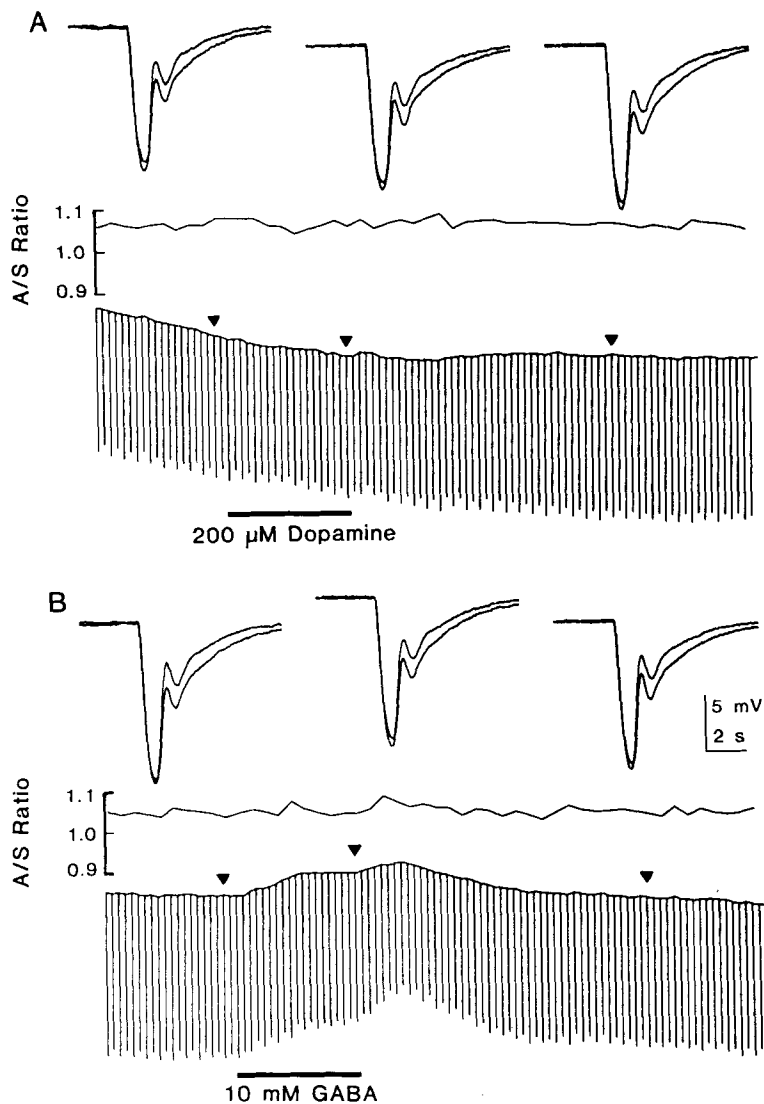


FIGURE 4. The effect of dopamine (*A*) and GABA (*B*) on the receptive field properties of skate horizontal cells. Spots (0.5 mm) and annuli (0.8 mm i.d., 5 mm o.d.) were presented alternately (at 30-s intervals) after adjusting their intensities so that both produced nearly equivalent responses. The upper traces in each half of the figure are recordings obtained at the times indicated (*triangles*) before, during, and after drug application (*heavy bar*), and show that the response waveforms were not affected by these agents. The bottom traces show plots of the peak light responses superimposed on measurements of the resting potential obtained during the course of the experiment; in this and subsequent figures the initial potential is in response to the annular stimulus. The middle traces chart the A/S ratio, i.e., the ratio of the responses obtained to annular vs. spot stimulation. An alteration in the receptive field size would be expressed as a marked change in this ratio (Dong and McReynolds, 1991). However, the fact that the A/S ratio remained relatively constant is an indication that no significant changes in receptive field organization were observed after application of 200  $\mu$ M dopamine (*A*) or 10 mM GABA (*B*). The high concentration of GABA did induce a membrane depolarization of  $\sim$ 5 mV, which probably reflects the activation of an electrogenic transport mechanism for GABA in these cells. Calibration bars are for the waveforms shown in the upper half of each figure.

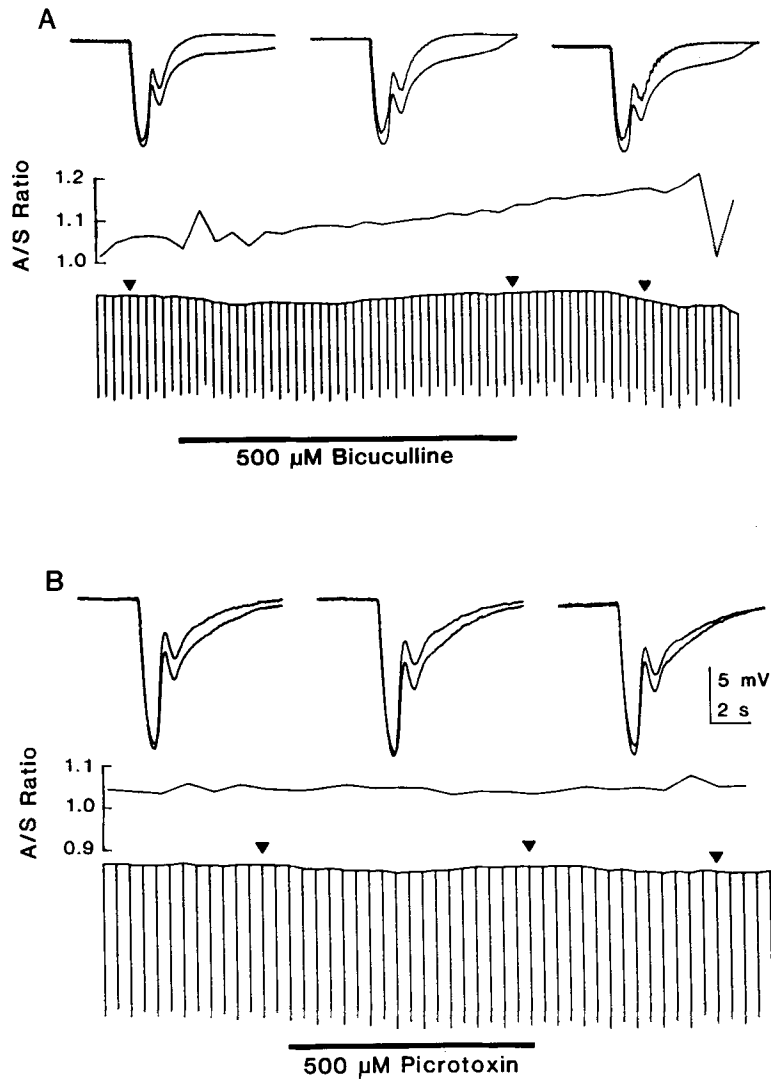


FIGURE 5. The effect of bicuculline (*A*) and picrotoxin (*B*) on the receptive field properties of a skate horizontal cell determined with alternating spot and annular stimuli (see Fig. 4 for details). No significant change in the resting potential was detected in the presence of 500  $\mu\text{M}$  bicuculline (*A*) or 500  $\mu\text{M}$  picrotoxin (*B*), nor were there any detectable changes in the A/S ratios; i.e., there were no significant changes in receptive field organization.

cluster of particles arranged in a more-or-less hexagonal array; the particles are  $\sim 7$  nm in diameter and usually remain attached to the protoplasmic leaflet or P face, with corresponding pits in the external leaflet or E face.

The gap junctions of skate horizontal cells were examined in EM micrographs of freeze-fracture replicas; the membranes of the horizontal cells were readily identified by their large extent and their location within the retina. Fig. 6 shows the typical

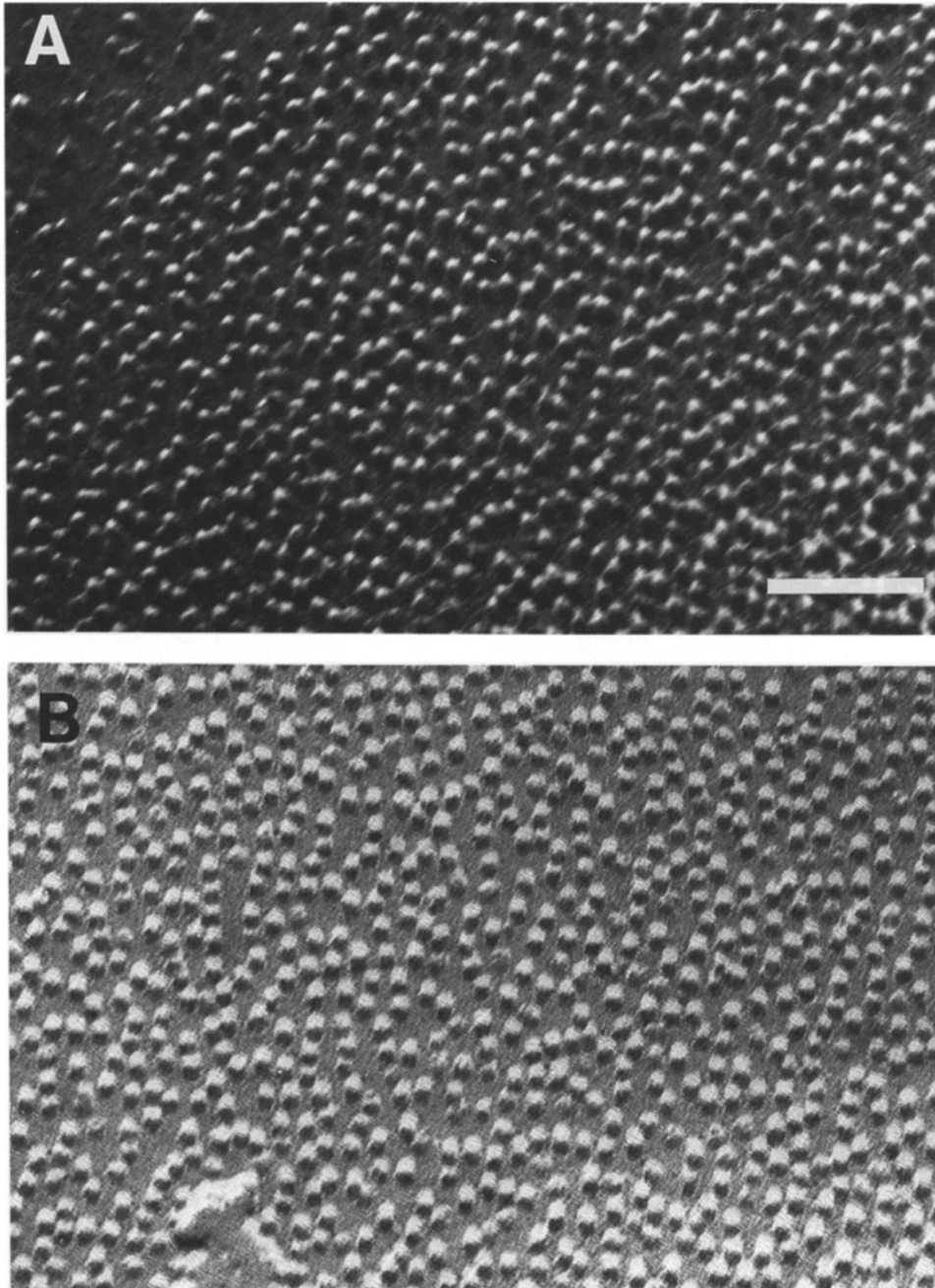


FIGURE 6. Electron micrographs of freeze-fracture replicas of skate horizontal cell gap junctions. Samples were prepared from both the dark- (*A*) and light-adapted retina (*B*). The density of junctional particles in this pair is  $3,000/\mu\text{m}^2$  for the dark-adapted preparation and  $3,200/\mu\text{m}^2$  for the light-adapted retina. Bar, 100 nm.

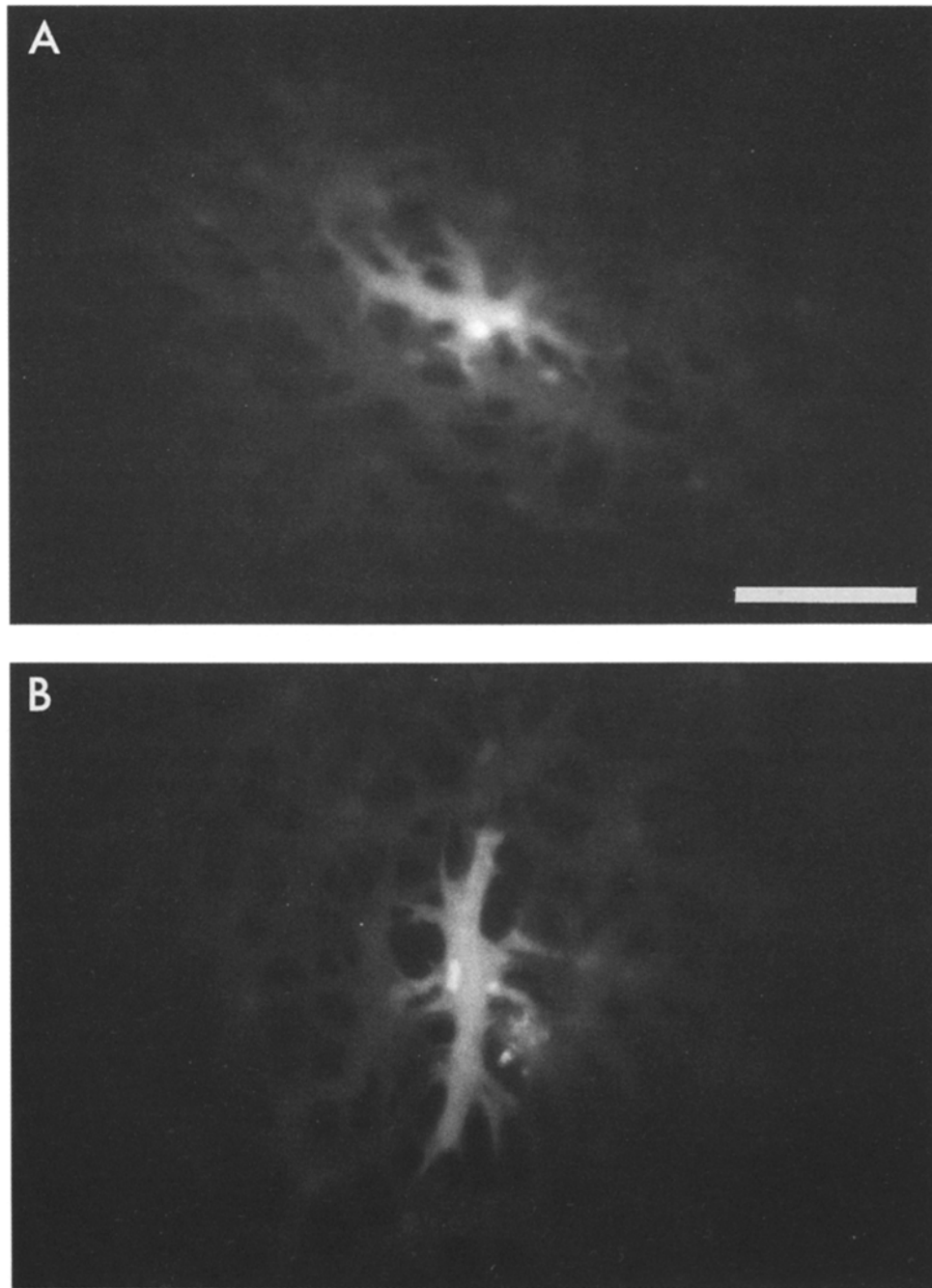


FIGURE 7. The effect of light adaptation on dye coupling between horizontal cells of the skate retina. Lucifer Yellow was injected iontophoretically into the cells by the application of hyperpolarizing current pulses (10 nA) for 15 min in dark- (*A*) and light-adapted (*B*) preparations, respectively. The dye was presumably injected into the cell in the center of the micrograph that shows the brightest fluorescence. The dye diffused into a large number of surrounding cells in both cases; i.e., dye coupling was not affected by the adaptive state of the retina. Calibration bar, 50  $\mu\text{m}$ .

appearance of the P face of the gap-junctional membrane of skate horizontal cells; the preparations were obtained from both dark- (*A*) and light- (*B*) adapted retinas. In dark-adapted preparations, the average gap-junctional particle density was found to be  $3,184 \pm 286/\mu\text{m}^2$  ( $n = 8$ ). Values for the particle density of light-adapted retinas were  $3,073 \pm 494/\mu\text{m}^2$  ( $n = 11$ ). Thus, no differences in the structural organization of the gap junctions could be distinguished based on the adaptive state of the retina.

#### *Dye Coupling of Skate Horizontal Cells in Dark- and Light-adapted Retinas*

The sizes of the gap-junctional channels are  $\sim 1\text{--}1.5$  nm, allowing small molecular weight fluorescent dyes to pass from one cell to another through the channels. This feature (dye coupling) has been used both to estimate the extent of coupling between cone-driven horizontal cells and to demonstrate the uncoupling effects mediated by either light adaptation or by the application of dopamine (Teranishi et al., 1984; Negishi et al., 1985; Piccolino et al., 1987; Tornqvist et al., 1988).

Once again, the rod-driven horizontal cells of skate gave no evidence for photic modulation of electrical coupling. Fig. 7 *A* shows that in the dark-adapted retina, the fluorescent dye Lucifer Yellow diffuses intercellularly through an extensive network of coupled horizontal cells; similar results were obtained in 16 experiments. This is consistent with the large size of the receptive fields measured electrophysiologically in skate and other species. However, in contrast to the results reported for cone-driven horizontal cells of the teleost, in which dye spread is markedly changed by light adaptation (Tornqvist et al., 1988; Baldrige and Ball, 1991; Umino et al., 1991), the spread of Lucifer Yellow was equally extensive in preparations that had been illuminated continuously with a  $-5\text{-D}$  background light (Fig. 7 *B*); similar results were obtained in 20 other cells in which the dye was iontophoresed under light-adapted conditions.

#### DISCUSSION

The very large receptive fields of skate horizontal cells, extending  $> 4$  mm across the retina (Figs. 2 and 3; cf. Dowling and Ripps, 1971), far exceed the dendritic arborization of the horizontal cell processes. This feature, together with the extensive dye coupling between neighboring cells seen with Lucifer Yellow (Fig. 7), and the freeze-fracture images of membrane particle arrays resembling those seen typically at electrical synapses (Fig. 6), provide strong evidence that skate horizontal cells are well coupled to each other via electrical (gap) junctions. Consistent with this interpretation is an earlier report of gap-junctional particles on skate horizontal cell membranes (Fain, Gold, and Dowling, 1976), and the results obtained with simultaneous recordings from pairs of horizontal cells in dogfish (*Mustelus*) retina that demonstrate directly the electrotonic spread of current between the external horizontal cells of this elasmobranch (Kaneko, 1971). Nevertheless, it is important to note that in skate (Ripps, H., unpublished observations), as well as in other species, photoreceptors often make electrical synapses with one another (cf. Raviola and Gilula, 1973; Witkovsky, Shakib, and Ripps, 1974; Schwartz, 1975; Fain et al., 1976). However, the contribution of such connections to the size of the horizontal cell receptive field appears to be relatively minor (Baylor, Fuortes, and O'Bryan, 1971; Schwartz, 1976;

Detwiler and Hodgkin, 1979; Copenhagen and Owen, 1980; Gold, 1979; Attwell and Wilson, 1980; Griff and Pinto, 1981; Attwell, Wilson, and Wu, 1984), and to a first approximation the size of the receptive field may be taken as an indication of the broad extent of electrical coupling between skate horizontal cells.

The fact that the receptive fields of the rod-driven horizontal cells of skate were unaffected by large changes in background illumination (Fig. 3) stands in sharp contrast to the results obtained for the cone-driven horizontal cells of other species, where the adaptive state of the retina is a major factor in governing the size and center-surround organization of the receptive field (Mangel and Dowling, 1985, 1987; Shigematsu and Yamada, 1988; Baldrige and Ball, 1991; Umino et al., 1991). This difference is also reflected in the constancy of the gap-junctional particle densities (Fig. 6) and intercellular dye diffusion (Fig. 7) in light- and dark-adapted retinas. In the cone-driven horizontal cells of most species in which these parameters have been examined, both dye coupling and connexon density exhibit changes concordant with the light-induced changes in receptive field organization; e.g., a light-induced decrease in intercellular coupling is often accompanied by a reduction in dye diffusion among neighboring cells and a decrease in connexon density (cf. Wolburg and Kurz-Isler, 1985; Baldrige et al., 1987; Kurz-Isler and Wolburg, 1986, 1988; Baldrige and Ball, 1991; Umino et al., 1991; Washioka, Watanabe, Negishi, and Tonosaki, 1991).

The stability of the electrical junctions of skate horizontal cells under various light-adapting conditions raises the possibility that maintenance of a constant receptive field is a fundamental requirement for rod-mediated pathways. However, Villa et al. (1991) reported recently that the receptive fields of rod-driven horizontal cells of the carp and dogfish (*Scyliorhinus*) can, in fact, be modified by background illumination. Surprisingly, their data indicate that light adaptation broadens the horizontal cell receptive field, and that it is significantly narrower in the dark-adapted retina. Despite taking special precautions to ensure that our preparations were initially fully dark-adapted, we were unable to detect any effect of light adaptation; skate horizontal cells exhibited only very broad receptive fields. Similar results (not shown) were obtained with intermittently illuminated adapting fields such as those used by Villa et al. (1991). It should be noted, however, that the skate differs from both carp and dogfish in that its retina contains only rod photoreceptors, whereas the retinas of the other species contain both rods and cones (albeit very few in *Scyliorhinus*). It may be that the same evolutionary pressures that have shaped the photoreceptor population of the skate retina and its unique adaptive properties (Dowling and Ripps, 1972; Cornwall, Ripps, Chappell, and Jones, 1989) have also acted to ensure the stability of the receptive field organization in this species. Alternatively, if a neural mechanism exists for modulating the receptive fields of cells in the rod pathway of the skate retina, it may occur at a later stage of information processing, e.g., in the inner plexiform layer (Jensen and Daw, 1986) or perhaps more centrally.

Not only are the electrical junctions between rod-driven horizontal cells of skate resistant to changes in the level of ambient illumination, they also appear to be unaffected by any of the pharmacological agents that have proven to be potent modulators of electrical coupling between cone-driven horizontal cells. Although we



did not test an extensive panel of putative neurotransmitters, the results obtained with dopamine, GABA and its antagonists, and glycine—compounds that appear to serve as transmitters for interplexiform cells in various species—gave no indication that any of these substances altered the electrical coupling between skate horizontal cells. For example, bathing the retina in 200  $\mu\text{M}$  dopamine (10–20 times the concentration that is typically used), which reduces the electrical coupling and greatly constricts the receptive field size of cone-driven horizontal cells of teleost, turtle, and mudpuppy (Piccolino et al., 1987; Yang et al., 1988; Dong and McReynolds, 1991), had no detectable effect on the receptive field properties of skate horizontal cells (Fig. 4A). This is perhaps not too surprising. First of all, if gap-junctional communication between horizontal cells is modulated by the activity of interplexiform cells, the skate retina appears not to have dopaminergic interplexiform cells (Bruun et al., 1984). Second, it appears that, even in retinas with dopaminergic interplexiform cells, dopamine is not a very effective modulator of electrical coupling between rod-driven horizontal cells; Mangel and Dowling (1987) and Tornqvist et al. (1988) have noted that the effect of dopamine on rod-driven horizontal cells of the perch retina was much smaller than that observed for the cone-driven horizontal cells of that animal.

The inhibitory amino acid GABA has also been shown to exert a profound effect on the horizontal cell receptive fields of turtle (Piccolino et al., 1987) and carp (Negishi et al., 1983). However, in these instances the GABA effect is indirect, acting on the horizontal cell through dopaminergic interneurons. Nevertheless, the observation that neither GABA nor its antagonists bicuculline and picrotoxin had any effect on the receptive field of skate horizontal cells (Fig. 5) is of particular interest considering the evidence that GABA may be the neurotransmitter utilized by interplexiform cells of the skate retina. The possibility that GABA did not reach the horizontal cells due to uptake by glial cells (cf. Ripps and Witkovsky, 1985) seems an unlikely explanation for its lack of effect; 10 mM GABA produced a clearly detectable depolarization of the horizontal cells (Fig. 4A), presumably reflecting activation of the electrogenic transport mechanism for GABA described previously by Malchow and Ripps (1990). Thus, our findings suggest that the GABA-ergic interplexiform cell in the skate retina is probably not involved with the modulation of horizontal cell receptive fields; it will be of interest for future investigations to determine what role can be ascribed to GABA-ergic interplexiform cells.

In sum, the results of this study demonstrate that there are fundamental differences between the gap-junctional properties of electrically coupled, rod-driven horizontal cells of skate and those of the cone-driven cells of other species. It is not possible, based on the available evidence, to identify the molecular basis for this distinction, but there is clearly the possibility that more than one class of electrical synapse is to be found on vertebrate horizontal cells. This would not be a unique situation; e.g., two types of gap-junctional proteins (connexin 32 and connexin 26) have been purified from hepatic cells (Nicholson, Dermietzel, Teplow, Traub, Willecke, and Revel, 1987). Although all gap-junctional proteins cloned and sequenced thus far share a common structural motif, and appear to derive from a superfamily of gap-junctional genes (cf. Beyer, Goodenough, and Paul, 1988), important differences have been reported with regard to their structural domains,

phosphorylation sites, and gating properties (cf. Bennett, Verselis, White, and Spray, 1988; Yancey, John, Lal, Austin, and Revel, 1989; Saez, Nairn, Czernik, Spray, Hertzberg, Greengard, and Bennett, 1990; Werner, Levine, Rabadan-Diehl, and Dahl, 1991). Unfortunately, far less is known about neuronal gap-junctional proteins (Bennett et al., 1991), and it would be unwise to speculate as to whether a difference in protein structure or the lack of some regulatory protein is responsible for the striking differences in coupling behavior of rod- and cone-driven horizontal cells.

We are grateful to Dr. R. Paul Malchow for his advice throughout the course of this work, and for his comments on an earlier draft of the manuscript. We thank also Dr. Henry Brown for help with the freeze-fracture preparations, and Jane Zakevicius, M.Sc. for assistance during the preparation of the manuscript.

This research was supported by grants (EY-06516 and EY-01792) from the National Eye Institute, USPHS, by a grant from the Illinois Eye Fund, and by an unrestricted award to the Department of Ophthalmology and Visual Sciences from Research to Prevent Blindness, Inc., New York, NY. H. Ripps is a Research to Prevent Blindness Senior Scientific Investigator.

*Original version received 23 January 1992 and accepted version received 21 April 1992.*

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