The Internal Horizontal Cell of the Frog

Analysis of Receptor Input

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The inner horizontal cell (IHC) of the retina of *Rana pipiens* was studied by light and electron microscopy of Golgi stained or horseradish peroxidase injected cells. Responses of IHCs were recorded with intracellular electrodes. Both axon and dendritic terminals of the IHC make synaptic contact with all classes of receptor. The terminals occur as lateral or medial processes at synaptic ribbons. Reciprocal invaginations of receptor into horizontal cell process are common. Different classes of receptor are contacted in about the proportions with which they occur in the retina. No tendency for contacts of one type of receptor to occur on a particular part of the cell was found. The IHC generates L-type S-potentials. Both rod and cone input is evident in the waveform of the response, its spectral sensitivity, and the effect on it of adaptation. Invest Ophthalmol Vis Sci 25:1382-1394, 1984

Two classes of horizontal cell have been described in the retina of the frog.12 An inner horizontal cell (IHC) with short thick dendrites forms a continuous layer at the outer border of the inner nuclear layer. The layer is penetrated at regular intervals by the processes of bipolar and Müller cells, giving the appearance of a perforated membrane, the “membrana perforata” of Krause.3 The inner horizontal cell, viewed from the side, looks like an inverted hat with a very prominent crown, which contains the nucleus and descends well down into the inner nuclear layer. The position of the nucleus of the IHC is internal to that of the outer horizontal cell and justifies retention of Cajal’s1 appellation rather than the nonspecific H1 and H2 terminology suggested by Stephan and Weiler.2 The outer or external horizontal cell (OHC) has long slender dendrites that extend through the outer plexiform layer, forming the meshwork of fibers that Krause3 called the “membrana fenestrata.” Cajal1 observed long processes, which he believed were axons in association with both types of cells.

The synaptic input to the IHC, with which this report is concerned, is mediated by filamentous bulb-tipped terminals; these rise from the dendrites and axons to the layer of receptor pedicles, which they penetrate. As in other amphibia, many of the dendritic terminals of the IHC form lateral processes at synaptic ribbons.4 Conventional synapses also have been observed among processes of horizontal cells in the outer plexiform layer of the frog.5 The nature of the synaptic contacts of these cells has not been previously reported in *Rana*. Studies in *Xenopus*, a more primitive anuran, have revealed a single class of horizontal cell6 that appears to be morphologically similar to the IHC of *Rana*. This cell contacts both rods and cones with its dendrites and axon terminals.6 The retina of *Xenopus*, however, differs in several respects from that of the more recent toads and ranid frogs. Its photopigments are based on vitamin A2 in contrast to the A1 based pigment of *Rana* and although it has two rod pigments, it has only a single cone pigment and that absorbs at 612 nm.7 Ranids and bufonids have two cone pigments absorbing at 502 and 580 nm (see below).

The electrical responses of horizontal cells have been described in many forms, but relatively few reports have dealt with frogs. The common horizontal cell responses of the frog, called luminosity or L-type S-potentials, are hyperpolarizing to all wavelengths of light and are known to originate in the IHC.4,6,8 Chromaticity or C-type S-potentials that hyperpolarize to short wavelength and depolarize to long wavelength stimulation were observed in *Rana catesbiana* by Naka et al.9 They did not identify the cells recorded, however, and it is possible that they actually penetrated chromatic bipolar cells.10

Although the literature contains many references to ranid frog receptors, the nature of receptor input to horizontal cells has not been described in this form. Also, spectral response studies of ranid IHC have not been published so functional evidence of
rod and cone input to the cells is not available. Previous studies in fish\textsuperscript{11,12} and turtle\textsuperscript{13,14} have demonstrated the value of detailed morphologic data concerning receptor contacts as a basis for the interpretation of horizontal cell recordings. It is the purpose of this paper to describe the type and distribution of receptor contacts and spectral sensitivity of IHC in \textit{Rana pipiens}. These studies involve intracellular recording and reconstruction of Golgi and horseradish peroxidase (HRP) stained cells, serially sectioned and viewed by light and electron microscopy.

**Materials and Methods**

The data presented in this paper were drawn from a series of 51 well-impregnated retinas, over 100 intracellular recordings, and 12 successful HRP injections of IHC. All material was obtained from adult frogs. Identification of receptor types contacted by IHC was made in Golgi stained retinas from which five cells were studied completely by serial reconstruction. Electron microscopy (EM) studies were done on HRP injected cells since the morphology of the synaptic connections was superior to that of the Golgi stained cells. Spectral responses were collected incidental to the HRP staining to evaluate relative rod and cone contributions to the cell responses.

**Golgi Procedure**

Retinas were removed from dark adapted, hemisected eyecups. The tissue was mounted as a Tefla gauze sandwich, vitreal side down, on a glass slide.\textsuperscript{15} The Colonnier Golgi-Kopsch procedure was modified to provide more selective staining. The modification consisted of using a wash of 2.5\% glutaraldehyde in distilled water for 30 min after chromation. Staining was accomplished with a solution of 0.75\% silver nitrate in 2.5\% glutaraldehyde. In other respects, the procedure was done as described previously.\textsuperscript{15} The use of glutaraldehyde instead of distilled water in these solutions resulted in a reduced tendency for cells to stain in clumps, and there was less fading of the stain prior to dehydration. Impregnated retinas were dehydrated rapidly in alcohol-acetone, infiltrated with epon-araldite and mounted flat on glass slides under a heavily weighted plastic sheet. These retinas were sufficiently flat to permit examination with oil immersion through the full retinal thickness. Camera lucida drawings were prepared at a magnification of \( \times1000 \). Photomicrographs were obtained with a Zeiss photomicroscope III.

**Serial Reconstructions**

Serial reconstructions of IHC were accomplished as described by Leeper.\textsuperscript{15} Well-isolated cells were removed from the flat mount, re-embedded and cross-sectioned serially at 1 \( \mu \)m. Collection of long ribbons of these vertical sections on glass slides was facilitated by the use of a large boat attached to the glass knife. Following counterstaining with Richard-son’s stain, silver impregnated processes were black; neuronal processes were various shades of blue. Stained IHC processes which appeared to be within the terminal of a receptor were considered to be in contact with it.

An orthogonal reconstruction of the stained cell was prepared from camera lucida drawings of the cross-sections. A line was drawn along the external limiting membrane with a notation of the type of receptor contacted at each point along it. The line was thickened beneath areas where silver appeared in the cross-section. An outline of the cell in orthogonal projection was obtained by constructing an array of the lines from all of the sections, suitably spaced to represent the final magnification. The position of every receptor contacted by the IHC was appropriately indicated on the cell outline, then transferred to the camera lucida drawing.

**Terminology and Receptor Identification**

The frog has five types of receptor which contain three different visual pigments.\textsuperscript{16} The red rod and the accessory member of the double cone, both of which contain a 502-nm pigment, will be referred to as the 502-rods and the 502-cone. The green rod, containing a 433 nm-pigment, will be referred to as the 433-rod. The single cone and the principal member of the double cone, both of which contain a 580-nm pigment, will be referred to as the 580S- and 580P-cones, respectively. Classification of receptors from the 1-\( \mu \)m-thick serial cross-sections was based on criteria described below and by Nilsson.\textsuperscript{17} The 502-rods, which constitute over one-half of all receptors, have a darkly stained nucleus that sits astride the external limiting membrane, a thick inner segment and a long thick outer segment. Their long slender axon ends in a small conical synaptic terminal. The 433-rods, 9–14\% of all receptors, have a pale nucleus in the inner tier of the outer nuclear layer and a rather thick axon, which may run obliquely for a short distance. Their inner segment is long and thin, but their outer segment resembles the 502-rods. The junction of the inner and outer segments is consistently more scleral than that of the 502-rods, and this provides a reliable marker of the presence of the 433-rod. Double cones are recognized easily by their combined nuclei, which are usually adjacent to the terminals, associated with an oil droplet in the inner segment of the principal member, and characteristic...
and prominent paraboloid in the inner segment of the accessory member. They account for about 15% of all receptors. The 580S-cones, about 20% of receptors, also contain an oil droplet in their inner segments, but this landmark is close to the outer limiting membrane, while that of the 580P-cone is about 10 μm more scleral in position. The nuclei of the 580S-cones are intermingled with those of the 433-rods, but are larger and paler. Thus each class of receptor can be identified reliably in a series of sections.

One goal of this study was to determine the number and types of receptors contacted by the IHC. About 10% of contacts observed were ambiguous in that an obvious terminal could not be associated unequivocally with a particular receptor. The most common problem was in determining which member or whether both members of a double cone contacted a particular terminal. Also 433-rods inner segments were difficult to trace so that identification was often based on the presence of a single, vitreally placed nucleus associated with a scleral outer segment and the absence of an oil droplet.

Shrinkage was not controlled in this study and all measurements are presented without correction for it. Previous use of these procedures was shown to cause a linear shrinkage of about 12%.[8,10] Measurements were made directly from the camera lucida drawings.

Electron Microscopy

Electron microscopy of HRP stained cells involved osmication of the reacted, washed tissue in 1% osmium for 1 hr, dehydration in graded alcohols and embedment in epon-araldite. The retina was sectioned serially at about 100 μm on a sliding microtome to permit localization of the stained cell that was removed from the thick section and re-embedded for serial thin sectioning. Section ribbons were collected on special three-slot grids, stained with uranyl acetate and lead citrate, and viewed with a Zeiss 10-B EM.

Electrophysiologic Methods

Intracellular recording was accomplished in the eyecup preparation with HRP filled glass microelectrodes having an impedance of about 100–200 megohms. The eyecup was maintained at room temperature in an oxygenated chamber. Photic stimulation was obtained with a shuttered quartz-iodine source attenuated with a balanced neutral density wedge. Chromatic stimuli were obtained by interposing Balzer interference filters with a half-intensity bandwidth of about 10 nm. Maximum intensity of the chromatic stimuli was 100 erg cm⁻² sec⁻¹ delivered to the surface of the retina. Centered spots of varying size were obtained by imaging a calibrated iris diaphragm on the retina. Spots could be varied from 75–700 μm. Identification of S-potentials was based on waveform, depth in the retina (about 100 μm), uniform receptive field, and dependence of amplitude on spot size up to about 400 μm. The 502-rods were occasionally impaled, but could be distinguished easily from S-potentials on the basis of their small receptive fields, spectral sensitivity, and slowly decaying hyperpolarization. HRP injection was accomplished by combined electrophoresis and pressure. HRP histochemistry involved the standard DAB reaction.

The methods used in this study conform to the ARVO Resolution on the Use of Animals in Research.

Results

Morphology

The IHC were easily distinguished by their short, thick, primary dendrites, which gave the cell’s border a scalloped appearance (Fig. 1). One of the primary dendrites gave rise to a short axon. Axon terminals generally occurred as single or paired bulb-tipped filaments (Fig. 1A, arrow), scattered along the axon at intervals of 10–20 μm. No axon terminal arborization was seen in either the Golgi or the HRP stained material and, as concluded by previous workers,[1,2,6] it seems unlikely that the frog has such a structure. Rather, the axon seems to end abruptly with a few filaments at a distance of 50–200 μm from the cell. Axonal length was about one-half that observed in Rana temporaria by Stephan and Weiler.2 This probably represents species differences since the dendritic fields of the latter form are about twice as big as those of Rana pipiens.

Dendritic terminals were usually more elaborate than those of the axon and supported from four to eight bulb-tipped branches, which arose from a single primary filament (Fig. 1B, arrow). Simpler dendritic terminals, with only one or two bulbs, were also occasionally seen.

The type and distribution of receptors contacted by a single IHC was determined by light microscopy of 1-μm-thick serial sections. Photomicrographs of every third section from such a series are shown in Figure 2. In the retinal flat mount, the dendritic terminals appear to cluster around the periphery of the cell (Fig. 1), but in cross-sections, it is seen that the terminals occur also above the cell soma (Fig. 2C–E). Determination of the receptor type contacted by a given terminal required examination of several adjacent sections, but was usually not ambiguous. Contacts appeared as enlargements at the end of the terminal filaments, embedded within the cytoplasm of a receptor pedicle (Figs. 2A–D, I, J; and 3).
All receptor types were contacted by the IHC. This is shown in the fortuitous section of Figure 3, in which contacts with two 502-rods (r), two 580S-cones (c), a 433-rod (gr) and a double cone (dc) are shown.

Although the numbers of each receptor contacted by an IHC were obtained easily and directly from the serial sections, it was necessary to reconstruct the cell to appreciate the distribution of contacts on it. A typical study is shown in Figure 4. A well-isolated Golgi stained cell is shown as photographed in the whole mount and drawn by camera lucida in Figure 4A and B. The orthogonal reconstruction of the cell is shown in Figure 4C. Each line in this figure represents a section, darkened wherever silver was present. When these drawings are placed in an array, an outline of the cell, projected at right angles to the plane of the section is obtained. Since the identity of every receptor contacted by the IHC is available in the cross-sections, it is a simple matter to construct a map of receptor distribution, as shown in Figure 4D.

This cell contacted 71 different receptors: 11 502-rods, 16 double cones (×2), 14 433-rods, and 14 508S cones. It was unusual in the small number of 502-rods contacted. The results of similar studies on this and four other cells are shown in Table 1. The cells ranged in position on the retina from 0.7-4.2 mm anterior to the optic disc along the horizontal meridian. It is apparent that the proportions of different types of receptors contacted varied considerably from cell to cell, but that no regular variation with retinal position occurred. The numbers of receptors contacted by the IHC varied from 48-71.

Approximately 27% of receptors contacted by the IHC were 502-rods. The 433 rods, 580S-cones, and double cones made up 15%, 17%, and 20% of contacts, respectively. Since one member of the double cone contains a 580-nm pigment, 580-cones actually constituted 35% of all contacts. Cone contacts occurred with about the same overall frequency or were slightly more common than rod contacts.

Receptor contacts of the axon terminals of the IHC were identified for 18 axons. In each axon, only one pair of terminals was studied, due to the length of axon involved. Thus all contacts of a given axon were not identified. Of the 18 axon terminal pairs,
Fig. 2. Photomicrographs of every third section of a serial 1-μm-thick series of cross-sections through a Golgi-stained IHC. Multiple terminal boutons occur around the periphery and above the cell perikaryon (X1700).

Eight contacted a single receptor; the others contacted two receptors. All classes of receptor were contacted. Frequency of contacts was 502-rods: 32%; 433-rods: 18%; 580S-cones: 12%; 580P-cones: 19%; and 502-cones: 19%. Considering the variability of dendritic contacts, the frequency of contacts of IHC dendrites...
and axons with different types of receptor seems quite similar.

The fine structure of dendritic and axon terminal synaptic contacts was studied in serially sectioned HRP stained cells. Previous studies have shown that frog IHC dendritic terminals may be lateral processes at ribbons.4,19,20 Occasional IHC dendrites also occupied a medial position at the synaptic ridge beneath a ribbon. Many contained invaginations of receptor membrane (reciprocal invaginations4), and it was possible to determine the relative frequency of these contacts in the serially sectioned material. HRP-injected cells were completely stained, including the axons, and were easily followed in serial sections. Fixation was not optimal, but was adequate for visualization of postsynaptic membrane specializations.

A typical HRP-stained IHC is shown in the photomicrograph of Figure 5A, which was obtained from a 100-μm-thick retinal cross-section. Bouquets of dendritic terminals are indicated by small arrows, and the axon, mostly out of focus, by a large arrow. This cell was re-embedded and serially sectioned (310 sections) for EM. The EM appearance of the HRP is shown in Figure 5B. The darkly stained process is the typical donut profile of the reciprocal invagination of receptor cytoplasm into an HRP filled horizontal cell process4 in this case of an axon terminal. The adjacent ribbon is indicated by the arrow.

Manual reconstruction of the individual stained terminals made possible evaluation of the frequency and relation of the reciprocal invaginations to lateral processes and synaptic ribbons. Four dendritic and two axon terminals were reconstructed. The dendritic terminal of Figure 6A, from the pedicle of a 502-rod, featured four synaptic enlargements. Three of the enlargements were lateral processes at ribbons (R). The fourth, at the end of a thin secondary filament, contained two reciprocal invaginations not related to ribbons (arrows).
Fig. 4. Distribution of receptor contacts of an IHC revealed by serial reconstruction. A, Photomicrograph of the IHC viewed in a retinal whole mount. Small arrows indicate synaptic terminals; large arrow indicates axon. B, Camera lucida drawing of the cell. C, Orthogonal reconstruction from serial cross-sections. Each line represents one section and is thickened to indicate the location of silver in the section (see text). D, Receptor map. Locations of 71 identified receptors are indicated by symbol on the camera lucida drawing. SC: 580S-cones; DC: double cones; GR: 433-rods; and RR: 502-rods (x1300).
The axon terminals closely resembled the dendritic terminals. The axon terminal shown in Figure 6B was found in the pedicle of a 433-rod. It had a single bulbous enlargement, which contained a reciprocal invagination and was also a lateral process (Fig. 5B). This terminal had three other filamentous contacts, only one of which was a lateral process at a ribbon. As is usually the case with horizontal cell processes, they showed no other synaptic specializations within the pedicle.

**Electrophysiology**

Spectral responses of an IHC are shown in Figure 7. Light-adapted responses had a shorter latency and a more rapid onset and decay than dark-adapted responses. The latter responses, although slower, were larger in amplitude. The responses shown in Figure 7A and B were obtained with photopically and scotopically balanced intensities of 479- and 638-nm light. Balance was determined for each frog as the topically balanced intensities of 479- and 638-nm light required to evoke an equivalent b-wave in the ERG under conditions of light and dark adaptation. The response waveforms were typical of cone and rod input, and there was sufficient difference in amplitudes under light- and dark-adapted conditions to suggest differences in efficacy of the input from these receptors. The similarity of light- or dark-adapted waveforms obtained with either 479- or 638-nm light is better appreciated when the appropriate traces are superimposed (Figs. 7C,D). With dark adaptation, stimulation by 479-nm light produced a rod-type S-potential, which was slower than that obtained with 638-nm light. With light adaptation, stimulation with 638-nm light produced a cone-type S-potential that had a more pronounced after depolarization than occurred with 479-nm light. The amplitudes of the responses to red and blue light shown in Figures 7C and D are not identical, indicating that the scotopic and photopic balances, which were obtained for the ERG, were not perfectly applicable to the horizontal cell.

Figure 8 shows the spectral sensitivity of six IHC recorded under mesopic conditions, and a curve representing the spectral sensitivity of the ERG b-wave of *Rana pipiens*, recorded under mesopic conditions. Maximum sensitivity of the b-wave, and the IHC occurred at about the same wavelength, 555-560 nm. The peak sensitivity clearly represents a predominance of the 580-cones, which constitute only about 37% of the receptors contacted by the IHC. Several cells were held sufficiently long to test with chromatic adaptation to red light. This caused peak sensitivity to shift to about 520 nm, to reveal the 502-cone input. With dark adaptation of these cells, peak sensitivity was about 500 nm, reflecting the preponderance of 502-rods. The mesopic spectral sensitivity studies of Figure 8 showed substantial sensitivity below 500 nm, presumed to reflect input from the 433-rods. Thus the broad spectral sensitivity of the IHC is in agreement with the morphologic evidence for input to the IHC from all classes of receptor. The data points shown in Figure 8, obtained using a criterion of 65% of maximum, are independent of the criterion level since the amplitude-intensity curves obtained with the different spectral stimuli could all be fit with the same log-tanh curve.

The size of the IHC receptive field was evaluated in a mesopic state of adaptation by the use of centered spot stimuli of different diameters. As spot size was increased from about 75 μm, response amplitude also increased (Fig. 9). Maximum amplitude was achieved for most cells with spots of 400-500-μm diameter. Further increase in spot size was without effect. The influence of spot size on response amplitude was independent of spot chromaticity (Fig. 9). This suggests that rod and cone input to the IHC are of about the same spatial extent and efficacy.

**Discussion**

This study has shown that the dendritic and axonal terminals of IHC of the frog contact both rods and cones. Little difference in form was seen among these terminals, which were conventional lateral processes and occasionally medial processes at ribbons, although branching tended to be less pronounced in the axon terminals. Reciprocal invagination of receptor into horizontal cell process was common in both rod and cone contacts of dendrites and axons. The IHC generate L-type S-potentials, which show evidence of mixed rod and cone input and have uniform receptive fields approximately 10 times larger than the dendritic spread of the cell.

The IHC of anurans have a characteristic shape and there is little question concerning the identity of

### Table 1. Receptor contacts of reconstructed IHC

<table>
<thead>
<tr>
<th>Cell</th>
<th>mm/disc</th>
<th>502-rod</th>
<th>433-rod</th>
<th>580S-cone</th>
<th>Double</th>
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<tbody>
<tr>
<td>1</td>
<td>0.7*</td>
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<td>4.2</td>
<td>19</td>
<td>7</td>
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<td>9</td>
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<tr>
<td>Mean (±SD)</td>
<td>18 (4)</td>
<td>10 (3)</td>
<td>11 (4)</td>
<td>13 (2)</td>
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</tr>
</tbody>
</table>

* Distance of cell from optic disc in millimeters.
the "inner horizontal cells" described by Cajal, Pier-
antoni and Ogden, Ogden et al, Witkovsky and Powell, Rozemeyer and Stolte, or the IHC of this study and the H1 horizontal cells described by Stephan and Weiler. The only difference appears to be size, probably attributable to species differences. Hassin and Witkovsky found the IHC of *Xenopus* to be about 50 µm in dendritic spread. Stephan and Weiler

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**Fig. 5.** HRP injected IHC. A, Photomicrograph of the cell viewed in a 100-µm-thick cross-section. Small arrows indicate dendritic boutons; large arrow indicates the (out of focus) axon. B, Electron micrograph of a typical "donut profile" of a reciprocal invagination of the receptor into the stained IHC axon terminal, which was a lateral process at a ribbon (arrow) synapse in the pedicle of a 433-rod. N: nucleus of the rod (calibration: A, 10 µm; B, 0.6 µm).
describe dendritic spread of the HI cells of *Rana temporaria* to be 40–90 μm, but the dendritic spread of the IHC of *Rana pipiens* is only about 35 μm in longest diameter.8

Gallego25 has suggested a general classification of horizontal cells of Tetrapoda based on whether or not an axon is present and on the types of receptor contacted. Horizontal cells with short axons were of
two types: Type I, the cone-rod horizontal cell of mammals, contacts cones with dendrites and rods with its axon; type II contacts only cones. If this classification is extended to the frog IHC, a third type must be added: type III contacting both rods and cones with both dendrites and axons, a mixed rod-cone horizontal cell.

Mixed rod-cone horizontal cells bearing axons have been described in *Ambystoma*, *Xenopus*, and *Rana*. To the best of our knowledge, mixed rod-cone horizontal cells of this type have not been described in nonamphibians. Thus rod and cone pathways are segregated in different cells in the fish, and functionally isolated in different parts of the same cell in mammals. Reptiles appear to have both rod and cone input to their axon terminals, but only a single receptor type contacts the dendrites of the short axon cells.

An elaborate terminal arborization of the type seen in mammals has never been described in ranid or bufonid amphibians. Past studies however, involved Golgi silver impregnation, a procedure notorious for its tendency to fail in staining certain structures. Thus some doubt has remained concerning the existence of a specialized arborization in these forms. The failure of HRP injection to reveal an arborization considerably strengthens the conclusion that it does not exist. Many of our (unsuccessful) HRP injections were partly extracellular and stained all processes passing through the injection site. These processes could be traced radially in all directions; some led to typical IHC; some were clearly distal IHC axons from which occasional terminal filaments arose. The axons always ends abruptly with a few twigs, even less elaborate than the small arborization described by Rozemeyer and Stolte in *Rana esculenta*. Thus the HRP stained axons resemble those stained by the Golgi procedure, and it seems likely that the anurans lack an elaborate axon terminal arborization.

It is not surprising that the horizontal cell axons of the anurans should differ markedly from those of urodeles since these major orders of amphibia have probably evolved independently from a stegocephalian ancestor. The urodelian *Ambystoma* has an axon terminal that resembles that of the fish. It is an elongated, thick cylindrical process connected to the soma by the slender axon. Like the frog, both dendrites and axons of *Ambystoma* contact rods and cones.

Orthogonal reconstruction of a stained cell from serial sections is an effective method for identifying all the contacts of a cell. Each of the IHC studied with this method contacted 80–90% of the juxtaposed receptors. The same terminal frequently contacted both rods and cones. No ordered differences in the proportions of different types of contacts across the cell could be discerned. Although the smallest terminals contacted a single receptor, most contacted more than one receptor.

The receptor mosaic of the frog is a regular array of 502-rods in which the other receptors seem to be scattered at random. A regular pattern of 502-rod contacts on the IHC was not apparent in this study, and 20–30% of the 502-rods within the dendritic field of a cell were not contacted. All types of receptor contacted all regions of the cell, so segregation of classes of receptor to a particular part of the cell was not seen. Also, the five reconstructed cells were situated from 0.4 to about 4.2 mm from the optic disk but showed no obvious morphologic variation with retinal position.

Reciprocal invaginations were found in every dendritic and axonal terminal with branches. These peculiar junctions were thus much more common than previously reported. It was concluded previously, from considerations of volume conduction, that the reciprocal invagination could not be the site of elec-
trical coupling between receptors and horizontal cells. Also membrane specialization were not seen so it seemed unlikely that these junctions could support conventional synaptic transmission. Recent studies in the human, however, revealed reciprocal invaginations containing collections of vesicles and membrane specialization suggesting a conventional synapse (S. Fisher, personal communication). Thus these ubiquitous contacts may be an additional avenue for receptor-horizontal cell interaction. The paucity of vesicle collections in the frog reciprocal invaginations might be the result of their state of dark adaptation at the time of killing. Each receptor evagination has a narrow neck through which synaptic vesicles may have to migrate. Vesicle output during dark adaptation should be high and might result in depletion due to inability of the vesicles to traverse the evagination neck in sufficient volume to offset release.

The spectral sensitivity of the IHC is broad, consistent with combined rod and cone input. The waveform of the response changed dramatically under different conditions of light and dark adaptation. Also it was not possible to reproduce the response to short wavelength stimulation with the use of scotopically balanced long wavelength stimulation. Under scotopic conditions, the response showed a prolonged decay, typical of the rod receptor potential. With photopic stimulation, the response decayed rapidly in the manner of cones. In several cells, held long enough to test the effects of chromatic or dark adaptation, adaptation with red light shifted the peak towards the shorter wavelength of the 502-cones. Dark adaptation caused a Purkinje shift to 520 nm, due to the input of the dominant 502-rods, but there was elevated sensitivity below 502 nm consistent with some input of the dominant 502-rods, but there was elevated sensitivity below 502 nm consistent with some input of the dominant 502-cones. Although there was some variation in the mesopic sensitivity of IHC to red and blue stimulation, all had a peak sensitivity at about 556 nm. This was also the wavelength of maximum sensitivity of the mesopic ERG b-wave. It would appear that, under these conditions, the ERG accurately reflects the spectral responsiveness of the IHC. Taken together, these results provide clear functional evidence of the input of all receptor classes to the IHC and are in agreement with the morphologic findings.

The receptive fields of the IHC were uniform. Response amplitude reached a maximum value with spots of 400-500-μm diameter and further increase in spot size was without effect. Also stimulation with an annulus in the presence of a background showed uniformity in the receptive field. Thus no evidence of surround inhibition of the kind reported for the tiger salamander was seen. Receptive field size was somewhat larger than reported for Rana catesbiana, but responses were otherwise similar in the two forms. Gap junctions between horizontal cell processes were not seen in this study but have been described in Xenopus. The receptive field size of the IHC of the frog, as in other animals, greatly exceeded the extent of the cell dendrites. It seems unlikely that the axon contributes much to this organization since it supports relatively few terminals and is rarely longer than 200 μm. Thus the basis of the large receptive field is probably a combination of electrical and conventional synaptic contacts among the IHC.

Key words: frog, horizontal cell, electron microscopy, serial reconstruction, S-potential

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