sis of ribonuclease A, glyceraldehyde-3-phosphate dehydrogenase, and lysozyme corresponds to ± 10 percent of the known mole percentage of aspartic acid in these proteins. For insulin A, however, which contains only asparagine, 70.4 percent of the asparagine was released as aspartic acid, indicating that considerable deamidation had taken place. Thus, the mole percentage of aspartic acid released from shell proteins should be regarded only as an approximation of the amount of aspartic acid present relative to asparagine.
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Goldfish Retina: Functional Polarization of Cone Horizontal Cell Dendrites and Synapses

Abstract. *In serial electron micrographs we observed that dendrites of goldfish cone horizontal cells are either central or lateral in ribbon synaptic triads, depending on cone and horizontal cell type. The chromatic properties of cone horizontal cell responses may be explained if the cone horizontal cells act as interneurons, receiving from cones through their central processes but acting on cones through their lateral processes.*

The initial step in the processing of neural information by the vertebrate retina involves interactions between photoreceptor, horizontal, and bipolar cells at the outer synaptic (or plexiform) layer. It is known that photoreceptors make synapses on bipolar cells and that horizontal cells modify bipolar cell responses (*1*).

Studies of synaptic ultrastructure in the retina (*2, 3*) have shown that the synapses of photoreceptors are characterized by clusters of postsynaptic processes opposite presynaptic organelles (synaptic ribbons). In goldfish, the postsynaptic processes are arranged in groups of three (*4*). Such triads comprise a bipolar cell dendrite that is centrally located opposite the ribbon and a pair of horizontal cell processes that are lateral to the ribbon and bipolar cell dendrite. We now report on the relation of the structural arrangements in ribbon synapses to functional pathways that involve cones and horizontal cells.

Stell and Lightfoot (*5*) identified three morphologically distinct types of cone horizontal cells in the goldfish retina by silver chromate impregnation (Golgi method). By morphological identification of the cones that made contact with processes of these cells, and by correlation of cone structure with visual pigment content as determined by microspectrophotometry (*6*), they showed that type H1 cells make contact with red-, green-, and blue-sensitive cones, H2 cells make contact with green- and blue-sensitive cones, and H3 cells make contact only with blue-sensitive cones.

Cone horizontal cells of all three types were selected from Golgi preparations, remounted, and sectioned serially at 60 to 90 nm in the plane perpendicular to the long axis of the cones. Fifty-three cone pedicles (synaptic endings) were sectioned serially and recovered. Each section was examined and photographed in the electron microscope. The series of micrographs were evaluated with regard to (i) cone type, identified by continuing the series of sections to the characteristic cone inner segments; (ii) number of cone synaptic ribbons; (iii) number of ribbon synapses in which a stained horizontal cell process made contact (*7*); and (iv) position of that contact (central or lateral) in the synaptic ribbon complex. Representative synaptic complexes were reconstructed graphically as projection drawings on orthogonal planes.

Electron micrographs of cone pedicles contacted by Golgi-stained horizontal cell processes (Fig. 1) demonstrate that horizontal cells of different types contact cones of a given type in different ways. Figure 1A shows dendrites of an H1 cell that terminate as lateral elements in synaptic ribbon complexes of a green-sensitive cone. This

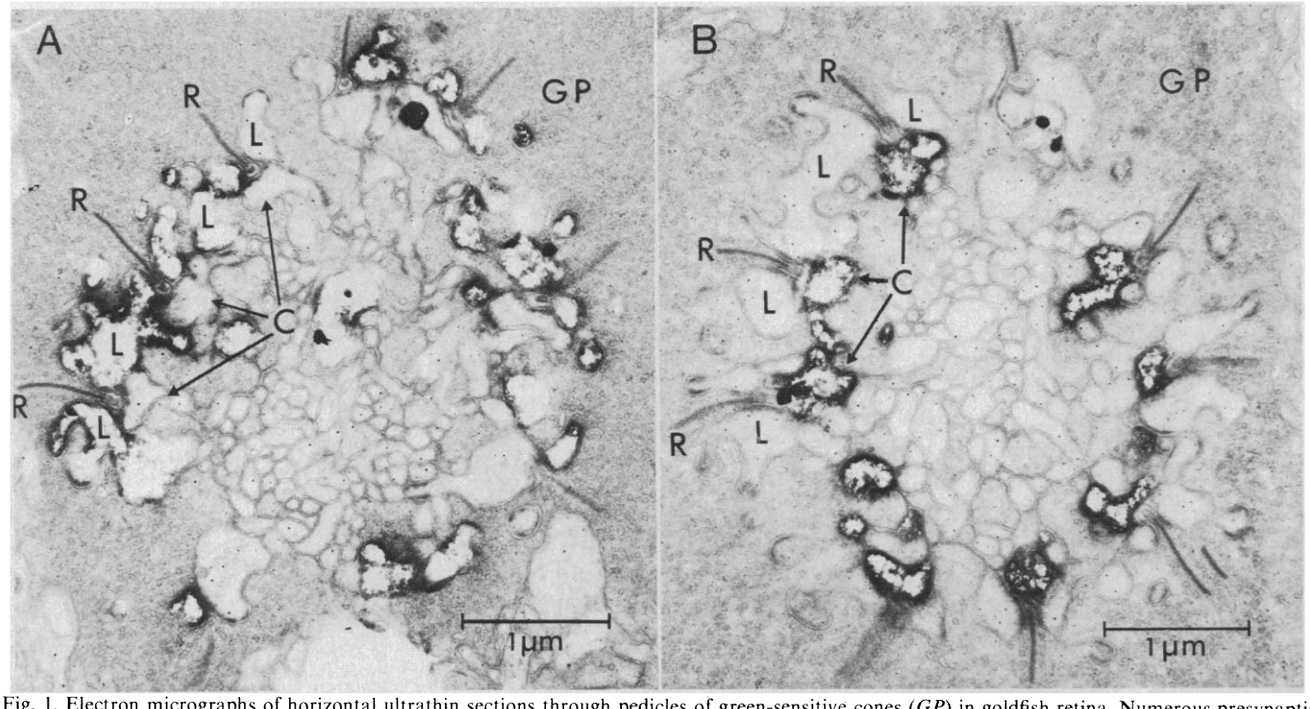

Fig. 1. Electron micrographs of horizontal ultrathin sections through pedicles of green-sensitive cones (*GP*) in goldfish retina. Numerous presynaptic ribbons (*R*) and postsynaptic triadic arrangements of central (*C*) and lateral (*L*) horizontal cell processes are shown. (A) Dendrites of Golgi-impregnated H1 cells are lateral in distribution. (B) Dendrites of Golgi-impregnated H2 cells are central in distribution.

mode of contact was expected (*2–4*). In contrast, Fig. 1B shows dendrites of an H2 cell that are also in contact with a green-sensitive cone; here the mode of termination was central. The contacts of these and other combinations of cones and horizontal cells are summarized numerically in Table 1 (*8*). These data indicate that the dendrites of horizontal cells do not universally occupy a lateral position in the ribbon synapse as was previously believed (*2–4*). Rather, the position of contact is a function of both the horizontal cell type and the cone chromatic type. The specificity of contacts of horizontal cell dendrites as lateral or central at cones of a given type suggests that the two modes of contact perform two distinct functions. Dendrites of bipolar cells in the central position are generally believed to be postsynaptic to the photoreceptors, receiving information from the cones but performing no known feedback function. We assume that horizontal cell dendrites also are exclusively postsynaptic to cones when they are located centrally in triads. Horizontal cell processes that terminate laterally in triads make the vast majority of their elaborately sculptured mutual contact with cones at a distance from both the synaptic ribbon and the central member of the triad complex (*4*). Since synaptic transmission from the cones is believed to occur near the apex of the ridge that contains the synaptic ribbon (*3*), we assume that processes which terminate laterally are not primarily postsynaptic to cones, but rather are presynaptic to cones. This is supported by the observation that H1 and H2 cells, which make extensive lateral contact with blue-sensitive cones (Table 1), have not been shown to receive significant functional input from them (*9*). Electrophysiological data on cones in the turtle (*10*) and pike perch (*11*) indicate that inhibitory synaptic feedback from horizontal cells to cones may be a general phenomenon.

Figure 2 predicts the spectral sensitivity

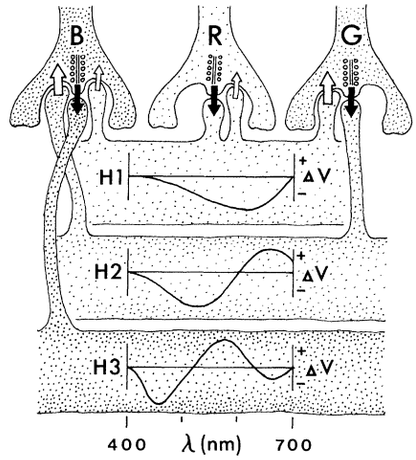

Fig. 2. Schematic representation of cone horizontal cell pathways in goldfish retina. Cone types are labeled *R*, *G*, and *B* (*6*). The H1, H2, and H3 cells generate monophasic, biphasic, and triphasic spectral response functions (*10*), respectively, by the pathways shown. Synapses where presynaptic and postsynaptic responses have the same polarity are shown by heavy arrows; probable minor synapses from cones to lateral processes, which contact the ribbon synapse in passing, are not indicated. Synapses where presynaptic and postsynaptic responses have the opposite polarities are shown by large and small outlined arrows; the synapses that are most instrumental in generating the spectral response functions (illustrated in the cell bodies) are indicated by the larger arrows.

functions of the horizontal cells (*12*) if the lateral contacts mainly represent inhibitory feedback from horizontal cells to cones, rather than excitatory synapses from cones to horizontal cells. This model also explains qualitatively the characteristic delays of the horizontal cell response components (*13*), in that the responses that show the longer delays are generated by the longer pathways. Finally, it accounts for the known chromatic inputs to H2 and H3 cells from cone systems with which they make no direct contact.

We have shown that horizontal cell dendrites in goldfish are structurally polarized and terminate in different positions in the ribbon synapses of different cones. We have concluded that the dendrites are functionally polarized according to these structural placements. Those dendrites that we believe to be presynaptic to cones do not, however, display the conventional synaptic ultrastructure seen in other short-axoned (Golgi type II) neurons (*14*). This raises the possibility that some neuronal interactions may be mediated by structures or mechanisms that are not known at present.

Table 1. Patterns of contact between identified cones and horizontal cells in goldfish retina, as revealed by Golgi–electron microscopy method. Ribbon synapses that were contacted by impregnated horizontal cells were simply counted, without weighting for the area of contact, and scored for prevailing mode (central or lateral in triads). Abbreviations: HC, horizontal cell; RS, ribbon synapse.

Cone HC		Cones contacted		Average number of RS contacted per cone HC	Distribution of contacts in ribbon synapses (percent of total)	
Type	*N*	Chroma*	*N*†		Central	Lateral
H1	1	R	9	86	12.8‡	87.2‡
		G	5	42	2.5	97.5
		B	2	4	0.0	100.0
H2	2	G	15	76	81.0	19.0
		B	6	24	8.5	91.5
H3	2	B	9	40	100.0	0.0

*See (*6*). †See (*8*). ‡Approximately two-thirds of these dendrites that terminated laterally also made extensive central contact in passing.

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7. The term contact refers here to a special proximity of a Golgi-impregnated horizontal cell process to the plasma membrane of a cone pedicle. Only processes that were part of a ribbon complex, very close (≤ 250 Å) to the cone synaptic membrane, and without intervening processes, were considered as making contacts.
8. Contacts of miniature single cones, which comprise < 10 percent of all cones in goldfish, are excluded from this account. While this does not significantly alter the quantitative data, the synaptic structure of these cones differs qualitatively from that of other cones. In Table 1 the numbers of cones contacted per H2 cell are about one-half, and cones contacted per H3 cell are about one-fourth, of the total expected (*6*). The (apparent) discrepancy is due to our failure to recover all the pedicles that were contacted by the larger horizontal cells in the serial ultrathin sections.
9. Electrophysiological data (*12*) have neither demonstrated nor excluded contributions of the blue-sensitive cones to the activity of monophasic and biphasic horizontal cells. Our morphological data suggest that blue inputs to H1 and H2 cells are present but very minor; they are excluded in this report so that the major pathways can be emphasized.
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