Rod Bipolar Array in the Cat Retina: Pattern of Input From Rods and GABA-Accumulating Amacrine Cells

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ABSTRACT

The potential and actual connections between rod and rod bipolar arrays in the area centralis of the cat retina were studied by electron microscopy of serial ultrathin sections. In the region studied there were about 378,000 rods/mm² and 36,000–47,000 rod bipolars/mm². The tangential spread of rod bipolar dendrites was 11.2 μm in diameter, and the “coverage factor” for the rod bipolar cell was 3.5–4.6. We estimate that about 37 rods potentially converge on a rod bipolar cell and that one rod potentially diverges to about four rod bipolar cells. The actual connections, however, are less than this by about half: 16–20 rods actually converge on a bipolar cell and one rod actually diverges to slightly less than two rod bipolar cells. The degree of convergence appears to reflect a compromise between the need to signal graded stimulus intensities (requiring wide convergence) and the need to maintain a good signal/noise ratio (requiring narrow convergence).

Amacrine varicosities that provide reciprocal contact at the rod bipolar dyad were studied in serial electron microscopic autoradiograms following intraocular administration of ³H-GABA or ³H-glycine. More than 90% of the reciprocal amacrine processes accumulated GABA in a specific fashion. This information, in conjunction with Nelson’s recordings from the rod bipolar and amacrine cells postsynaptic at the dyad (Nelson et al.: Invest. Ophthal. Mol. 15:946-953, '76; Kolb and Nelson: Vision Res. 23:301-312, '83), suggests that feedback at the rod bipolar output might be positive.

Key words: vision, gamma-aminobutyric acid, dark adaptation, feedback

The rod bipolar cell is a crucial link in the pathway which is thought to carry quantal signals from the rods to the ganglion cells (Barlow et al., '71; Mastronarde, '83; Kolb and Nelson, '83; Smith et al., '86):

rods → rod bipolar cell → A11 amacrine cell → cone bipolar axon → on-ganglion cell

&
off-ganglion cell

The pattern of convergence of rods onto the rod bipolar cell is likely to be important in determining the noise level in the rod bipolar cell and in subsequent stages along this pathway (Baylor et al., '84). Boycott and Kolb ('73) provided evidence from electron microscopy of Golgi-impregnated neurons that about 15 rods converge on a rod bipolar cell and that one rod diverges to about two rod bipolar cells. These observations, however, were made on rod bipolar cells of unspecified eccentricity. It seemed desirable, particularly since cell density and dendritic spread can vary with eccentricity (eg., Steinberg et al., '73; Boycott and Wassle, '74), to investigate in more detail the relationships between the rod and rod bipolar arrays in the area centralis.

The rod bipolar pathway is fitted with a feedback circuit at the rod bipolar axon terminal. At every synaptic ribbon where the rod bipolar axon contacts the A11 amacrine cell, it also contacts a second amacrine process ("dyad," Dowling and Boycott, '66). This second process, which can belong to any of at least four amacrine types (A6, A8, A13, A17; Kolb and Nelson, '83), invariably reciprocates a synapse back onto the rod bipolar axon (Kolb, '79; McGuire et al., '84). Since the rod bipolar axon has about 25 ribbons (McGuire et al., '84), it also bears about 25 feedback contacts that

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must provide a powerful modulatory effect on every rod signal that passes along the pathway. The somas of reciprocal amacrine A6, A13, and A17 have been shown to accumulate GABA and muscimol, a GABA analogue (Pourcho and Goebel, '85; Freed et al., '83; type A8, has been shown to accumulate glycine (Pourcho and Goebel, '85). It was unknown, however, whether these types account for all of the reciprocal synapses to the rod bipolar or only a small portion.

We have studied these issues in series of ultrathin sections through the area centralis of three retinas photographed in the electron microscope. Two of the series were prepared as autoradiograms following intravitreal injection of either \( ^3 \)H-GABA or \( ^3 \)H-glycine. We used this material to determine the pattern of convergence and divergence between the rod bipolar arrays and also to determine the patterns of transmitter accumulation by all the processes presynaptic to individual rod bipolars.

**MATERIALS AND METHODS**

We studied three retinas, each from a different cat. An animal anesthetized with pentobarbital (40 mg/kg) and perfused with a phosphate-buffered mixture of 2% glutaraldehyde-2% paraformaldehyde. The retina was osmicated, stained en bloc with uranyl acetate, dehydrated, and embedded in Epon. In one cat we had injected intravitreally \( ^3 \)H-GABA (100 \( \mu \)Ci, 40 Ci/mmol in 10 \( \mu \)l of saline) 3.5 hours before perfusion, and in a second animal we had injected \( ^3 \)H-glycine in a similar manner 1 hour before perfusion. The GABA-labeled retina provided series I: 110 silver-gold sections cut radially. The glycine-labeled retina provided series II: 400 sections cut tangentially through sublamina \( b \) of the inner plexiform layer. Series III, 279 silver-gold sections cut radially, had been prepared by E. Cohen (Cohen and Sterling, '86). The isotopically labeled series of sections were prepared as electron microscope (EM) autoradiograms (Ilford L-4 emulsion, 1-2 weeks exposure) as described previously (Davis et al., '79).

All series were photographed in a JEOL 120B microscope at an accelerating voltage of 120 kV and printed at a magnification of \( \times 2,000-7,000 \). Reconstructions were accomplished by tracing outlines from successive sections onto acetate sheets aligned on a cartoonist’s jig. The outlines were digitized and entered into a PDP 11/34 computer which controlled an X/Y plotter. The plotter drew a two-dimensional view of the neuron in any rotation with the hidden lines removed. For further technical detail consult Stevens et al. ('80). All three series have been used extensively in other studies (Freed et al., '83; Cohen and Sterling, '86).

**IDENTIFICATION OF THE ISOTOPICALLY LABELED COMPOUND IN THE TISSUE**

In the GABA-labeled retina, \( ^3 \)H-GABA has been shown to account for almost all of the radioactivity (Freed et al., '83). In order to identify the preponderant isotopically labeled compound in the glycine-labeled retina, we separated glycine from its tritiated metabolites. In two experiments an eye was injected with tritiated glycine. After 1 hour the eye was removed and the retina excised. The retina was rinsed three times for 15 minutes in ice-cold, phosphate-buffered saline, then homogenized in ice-cold 80% methanol, and centrifuged at 10,000g. The pellet from the first centrifugation was resuspended and centrifuged again. The supernatants from the first and second centrifugations were combined and an aliquot concentrated by evaporation with a stream of nitrogen. The concentrate, mixed with non-radioactive glycine, was spotted on plastic thin-layer chromatographic plates and the chromatograms developed in three different solvent systems. Glycine was visualized with ninhydrin, the plate cut into 1-cm strips, and the radioactivity in each strip measured with a scintillation counter. Greater than 90% of the radioactivity in the supernatant comigrated with authentic glycine; the pellet from the second centrifugation contained less than 2% of the radioactivity. Thus we conclude that more than 90% of the silver grains in the electron microscopic autoradiography of series II represent \( ^3 \)H-glycine.

**RESULTS**

**Rod and rod bipolar arrays**

In a radial series (III) we located every rod axon terminal ("spherule") present in a small patch of retina (Fig. 1A). There were 84 spherules in a 222-\( \mu \)m\(^2\) area, which gave a density for the rod array of 378,000 rods/mm\(^2\). This agrees with the rod density reported by Steinberg et al. ('73) at the same eccentricity, about 300 \( \mu \)m from the exact center of the area centralis. The mean nearest-neighbor distance in the tangential plane between the centers of rod spherules was 1.06 \pm 0.42 \( \mu \)m. The ratio between mean and standard deviation of this measure was about 2.5, indicating the rod spherule array was only moderately regular (Wässle and Riemann, '78; Sterling, '83).

In this same series, just deep to the identified rod spherules, we located all the rod bipolar cells present in the inner nuclear layer. These were identified by a dark cytoplasm and dendrites that terminated within rod spherules. There were 15 cells in an area of 418 \( \mu \)m\(^2\), which gave a density for the rod bipolar array of about 36,000/mm\(^2\).

In the tangential series (II), 300 \( \mu \)m dorsal to the center of the area centralis, we identified all the rod bipolar axons. These axons were recognized initially at the border between sublaminae \( a \) and \( b \) by their dark cytoplasm, and each was traced deeper into sublamina \( b \) in order to confirm that it assumed the club shape and connections characteristic of the rod bipolar cell (Fig. 3; Cajal, 1892; Kolb, '79; McGuire et al., '84). Thirty-seven axons were identified in an area of 790 \( \mu \)m\(^2\), which put their density at about 47,000/mm\(^2\). The mean distance to the nearest neighbor for the rod bipolar axons at the \( a/b \) border was 54,500/mm\(^2\), and the nearest-neighbor distance was 0.42 pm. The ratio between mean and standard deviation of this measurement is 2.1, indicating that at this level the array of rod bipolar axons is not strikingly regular (Wässle and Riemann, '78; Sterling, '83). However, the axon terminals deeper within sublamina \( b \) were more regularly arrayed: when the axon stalks were traced into sublamina \( b \), six of them (14%) branched to produce a pair of club-shaped terminals instead of the usual single one (see Cajal, 1892; Fig. 1C). The density of the array including these supernumerary terminals was 34,500/mm\(^2\), and the nearest-neighbor distance was 3.2 \pm 0.7 \( \mu \)m. The supernumerary terminals appeared to fill holes in the array making it more regular (mean/S.D. = 4.6) than at the \( a/b \) border.

**Potential connections between rod and rod bipolar arrays**

The dendritic arbors of three rod bipolar cells were reconstructed from series III in order to determine their tangen-
Fig. 1. Neuronal arrays (left) and their frequencies of nearest-neighbor distances (right). A: Tangential distribution of rod spherules in a patch of retina 300 μm from the center of the area centralis. Reconstructed from a radial series. The rod spherules lie in ranks of 2–3, which allows the tangential projection of their centers (dots) to approach closer than the rod spherule diameter (about 3 μm). The nearest-neighbor distances of rod spherules at the border of the array were not included because their nearest neighbors may have been excluded from the array. B: Array of 37 rod bipolar axons in a patch of retina 300 μm dorsal to center of area centralis. Tangential section through the axon "stalks" at junction of sublaminae a and b. This array exhibits a wide range of nearest neighbor distances. C: Same array of rod bipolars but tangentially sectioned through the axon terminal at the junction between sublamina b and the ganglion cell layer. Six of the axons produced a supernumerary terminal (circled). The axon terminal array exhibits a narrower range of nearest neighbor distances than the axon stalk array (compare left halves of B and C). A–C: The density of each array (see text) was derived by dividing the number of axons by the area indicated by the outline, which is one-half the nearest-neighbor distance from the outermost members of the array. All nearest-neighbor frequencies differed significantly from the frequencies expected from a random distribution ($\chi^2 = 27.3, 22.3, 55.7$, A, B, and C, respectively; $P<0.005$, df = 6) and more likely derive from a Gaussian distribution ($\chi^2 = 4.4, 3.9, 2.9$ for A, B, and C, respectively).
Fig. 2. Rod bipolar somas and dendritic arbors reconstructed from radial sections. A: Three rod bipolar dendritic arbors in tangential view. The circles touch the outermost points of the arbor and circumscribe the rod bipolar dendritic field. The dendrites appear not to meet in the center of the field because their common origin, the soma, is omitted. B: The same three rod bipolar cells in radial view. Circles represent rod spherules that contact the rod bipolar cells. Some rod bipolar dendrites (*) could not be followed to the level of the rod spherules, and thus more rod spherules than those indicated presumably contacted the rod bipolar.

To estimate the dendritic field area we drew a circle just touching the outermost dendritic tips. The dendritic fields were of modest extent (11.2 ± 0.1 μm diameter) with an area of 9.9 × 10⁻⁶ mm². This allowed us to calculate the "coverage factor" for the rod bipolar (dendritic field area × cell density; Wässle and Riemann, '78). Using the somewhat different rod bipolar densities found for the two series (37,000 and 47,000), we obtained a coverage factor of 3.7-4.7.

The coverage factor provides an estimate of the number of rod bipolar cells that one rod can reach (potential divergence), as distinct from the number of rod bipolar cells a rod actually does contact (actual divergence). Because a rod spherule's synaptic site represents a point on the retinal surface, the rod bipolar coverage factor implies that each rod can contact, on the average, 3.7-4.7 rod bipolars. The potential convergence of rods onto the rod bipolar cell can be estimated by multiplying the dendritic area of the rod bipolar dendritic field by the density of rod spherules in the region of retina studied here; this gives about 37 rods in the dendritic field of a rod bipolar cell.

Actual connections between rod and rod bipolar arrays

The most direct method of determining the actual connections between rods and rod bipolars would be to trace the dendrites of the reconstructed rod bipolar (Fig. 2) to their sites of contact with rods. By this method we were able to observe directly only 5-6 spherules per rod bipolar. This method clearly underestimated the actual convergence because it was difficult to follow the finest tips of the dendritic
thorns to their terminations. Therefore we estimated the actual convergence in another way.

The rod spherule contains two synaptic ribbons; each of these usually contacts a different rod bipolar cell although sometimes both ribbons contact the same cell (Boycott and Kolb, '73; Sterling et al., '87b). Thus the actual divergence must be slightly less than 2. The actual convergence can be calculated by the following reasoning: if each of the 378,000 rods in mm² of retina diverges to an average of two rod bipolar cells, then there are about 756,000 such synaptic connections. These connections are divided among 36,000–47,000 rod bipolar cells, so 16–21 connections must be apportioned on average to each rod bipolar cell. This estimate that 16–21 actually converge on a rod bipolar cell in the area centralis agrees well with about 15 rods contacting Golgi-impregnated rod bipolar cells of unknown eccentricity (Boycott and Kolb, '73). It is interesting that only about half of the connections that could potentially exist between the two arrays are actually realized.

In the preceding paragraph we derived the actual convergence of one array onto another from knowledge of the divergence and the densities. This reasoning can be formalized in an equation:

$$\frac{A}{B} = \frac{\text{conv}}{\text{div}}$$

where type a is presynaptic to type b; $A = \text{density of cell type } a$; $B = \text{density of cell type } b$; $\text{conv} = \text{average number of cell type } a \text{ presynaptic to individual type } b$ (convergence); and $\text{div} = \text{average number of cell type } b \text{ postsynaptic to individual type } a$ (divergence).

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Fig. 3. Electron microscope autoradiograms of rod bipolar synaptic connections. Silver grains represent accumulation of $^3$H-GABA. A: Rod bipolar cell (RB) with synaptic ribbons at dyad. Postsynaptic at the dyad are the A11 amacrine cell and the reciprocal amacrine cell (ra) that feeds back a chemical synapse onto the rod bipolar cell. Note silver grains in both ra profiles. B: Lower magnification showing the other types of processes contacting the rod bipolar cell. IPC, interplexiform cell; nr, nonreciprocal amacrine cell. Arrow at lower right marks large gap junction between an AII and a cone bipolar process.
Reciprocal amacrines
Nonreciprocal amacrines

AII amacrines and interplexiform cells (IPC)

Specifically labelled, heavy
Specifically labelled, moderate
Labelling not measurable

Reciprocal amacrines
Nonreciprocal amacrines

AII amacrines and interplexiform cells (IPC)

Specifically labelled, heavy
Non-specifically labelled, moderate
Labelling not measurable

Figure 4
Thus the ratio of densities should be proportional to the ratio of convergence to divergence. In addition, as pointed out to us by Dr. Heinz Wässle, convergence and divergence can be replaced in equation 1 by the numbers of contacts. Thus, for Equation 1, \( \text{cond} = \text{average number of contacts individual cell type a makes upon type b cells} \); \( \text{div} = \text{average number of contacts individual cell type b receives from type a cells} \).

We anticipate that this equation will be useful in other studies of connections between neuronal arrays; in particular, it can be used to check the accuracy of determinations of synaptic connections between arrays by comparison to their measured densities (Sterling et al., '87b).

**Transmitter accumulation at the dyad**

The typical structure of the dyad and its labeling pattern following intraocular injection of \(^3\)H-GABA are shown in an EM autoradiogram (Fig. 3A). A ribbon in the rod bipolar cell points between the AII amacrine cell and a reciprocal amacrine profile; only the reciprocal profile bears a silver grain. The amacrine profile at the bottom of this picture is also reciprocal because it is shown to be presynaptic to the rod bipolar cell in this section and was found postsynaptic to a ribbon in an adjacent section. This reciprocal amacrine cell also bears silver grains. The electron micrograph on the right (Fig. 3B) is a lower magnification and shows many profiles surrounding the rod bipolar axon. For the most part neither their identities nor their labeling intensities could be determined in a single section. Therefore, we reconstructed five rod bipolar axons and their synaptically associated processes from a radial series of sections (series II) about 200 \( \mu \text{m} \) from the center of the area centralis.

A reconstructed axon is shown in Figure 4A. It contained 17 synaptic ribbons, each presynaptic to the small varicosities of a reciprocal amacrine. Fourteen of the ribbons were also presynaptic to all processes. There were three large processes from three different AII amacrine cells, and each received contact at several dyads. Scattering along the rod bipolar cell's terminal club and part of its stalk in sublamina a, there were 11 amacrine varicosities each providing a single contact to the rod bipolar but receiving none in return (non-reciprocal). Near the \( a/b \) border two small varicosities of a different sort also provided a single contact each but received none in return. These varicosities were pale and were observed at higher magnification to have a synaptic clefts divided along the center by a dense line. These features were characteristic of the interplexiform cell which is known to contact the rod bipolar axon (Nakamura et al., '80).

There were very few silver grains overlying the rod bipolar cell itself (13 grains/100 \( \mu \text{m}^2 \)) or over the AII amacrine processes (9 \( \pm \) 2 grains/100 \( \mu \text{m}^2 \)). All of the reciprocal ama-
Fig. 6. The hypothesized effect of rod convergence on the signal/noise ratio of the rod bipolar and AII amacrine cells for a quantal event in one rod. One rod (hollow) contributes its signal and continuous noise (S/N = 5); the other 15 rods (filled) contribute only noise. Ignoring other sources of noise and calling the signal amplitude 5 and the noise amplitude 1, the signal/noise ratio would go from 5 in the rod to $5/\sqrt{16}$ or about 1.3 in the rod bipolar cell. The signal would tend to be additionally degraded by convergence at the next stage along the pathway. The AII amacrine cell receives input from about 26 rod bipolar cells (solid) in addition to the two which carry the signal rod (hollow). Thus the AII receives indirectly through the bipolar cells continuous noise from about 210 rods which would cause the signal/noise ratio within the AII to drop to $5/210$ or about 0.024. Any other sources of noise, such as synaptic noise in the rod bipolar cell or AII amacrine cell would reduce the signal/noise ratio even further, so this is a best-case estimation. With a thresholding mechanism at the outputs of the rods and rod bipolar cell the signal/noise ratio could be preserved despite the great convergence.

In the glycine experiment (series II) the rod bipolar cells had hardly any silver grains. Many of the AII amacrine processes, as expected (Pourcho and Goebel, '85; Cohen and Sterling, '86), accumulated silver grains above the nonspecific level (Fig. 5). However, the reciprocal amacrine varicosities did not accumulate silver grains. We studied their grain densities at 40 dyads in serial sections of this material without finding a single one that was specifically labeled.

**DISCUSSION**

**Convergence of rods onto rod bipolars**

In the region of the area centralis studied here, about 37 rod spherules would fall within the dendritic field of a rod cray processes that were large enough to permit accurate measurement of grain density contained many silver grains $(23 \pm 5$ grains/100 $\mu m^2)$. The grain density over the reciprocal amacrine cells showed a specific accumulation of GABA (Fig. 5). Several of the nonreciprocal varicosities (5/9) also exhibited specific accumulation. The two interplexiform varicosities, as expected (Nakamura et al., '80), showed intense accumulations of silver grains (10× nonspecific). These patterns were confirmed in four additional reconstructions (Fig. 4B–D). It thus appears that most (90%) of the reciprocal amacrine varicosities specifically accumulated GABA. In addition, about 40% of the nonreciprocal amacrines, as well as the interplexiform varicosities, were also GABA-accumulating.
bipolar cell if its dendritic field was exactly circular. Yet the dendritic field does not fill in the circle but forms an irregular and nonsymmetrical candelabra (Fig. 2). This explains why we found only about 16–21 rods actually converging by chemical synapses upon the rod bipolar. The functional convergence of rods onto the rod bipolar cell could conceivably be greater than the anatomical convergence of 16–21 because rod spherules can be electrically interconnected via gap junctions with cone pedicles (Kolb, '77; Smith et al., '86). Such coupling between rods would tend to reduce the signal produced by a quantal event in a single rod (Schwartz, '76). We think it likely, therefore, that at scotopic levels the rods are uncoupled (Smith et al., '86) and that the anatomical circuit accurately reflects the number of rods converging functionally onto the rod bipolar.

One might ask why the convergence should be restricted at this first stage of the circuit to only 16–20, since about 1,500 rods must ultimately converge on a beta ganglion cell (Sterling et al., '87b). The rod bipolar pathway is thought to serve the scotopic range (Smith et al., '86), where no rod receives more than one quantum within its summation time (Sterling et al., '87a). At these low intensities a single quantum appears in the ganglion cell as a burst of several spikes (Barlow et al., '71; Mastronarde, '83). This implies that the quantal event in the rod outer segment is transmitted to the ganglion cell without significant decrement. Yet Baylor et al. ('84), who measured the signal/noise ratio in the rod outer segment as 5, pointed out that the signal/noise ratio will tend to be degraded in the rod bipolar as the square root of number of rods converging. The signal/noise ratio would go in the present case from 5 in the rod to about 1.3 in the rod bipolar (5/√16; see Fig. 6). Thus a limited convergence of rods onto the rod bipolar, would help preserve the signal/noise ratio in the rod bipolar. In addition, according to Baylor et al. ('84), noise might be rejected at the rod = rod bipolar junction if this synapse had a threshold for effective transmission at a voltage more hyperpolarized than the voltages associated with noise.

The problem of noise accumulated by convergence arises again at the next stage along the rod bipolar pathway, where about 28 rod bipolars apparently converge on a single AII amacrine (Sterling et al., '87b). Thus the signal/noise ratio would drop by a factor of 5.3 at this synapse (√28). One can calculate that without any threshold mechanism at either synapse (rod = rod bipolar, rod bipolar = AII amacrine) the signal/noise ratio would drop from 5 in the rod outer segment to less than 0.35 in the AII amacrine (see Fig. 6). Thus the quantal signal will probably be extracted from the noise at the rod bipolar = AII synapse as well. The structure of the rod bipolar dyad seems suited to accomplish this, as described below.

The best signal/noise ratio in the rod bipolar would be achieved, of course, by a convergence of only 1 rod upon a rod bipolar cell. This would, however, leave the rod bipolar cell without a response graded to stimulus intensity. In the environmental intensity range below 1 log q/μm²/sec, where no rod gets more than 1 quantum within its summation time each rod produces a quantal voltage. With a convergence of 1 the signal/noise ratio would be optimum but the voltage in the rod bipolar would also be quantal, and the rod bipolar could not signal increases in this intensity range. With the convergence of 16–20 quantal events originating in different rods are be summed by the rod bipolar cell. Thus with increasing intensity, increasing numbers of rods produce simultaneous voltages in the rod bipolar soma, and the net voltage in the rod bipolar soma is proportional to intensity. We suggest that a rod = rod bipolar convergence of 16–20 is a compromise between the requirements for protecting the signal/noise ratio and for accomplishing spatio-temporal summation over the intensity range where rods produce quantal signals.

Feedback at the dyad

We have noted that every feedforward synapse of the rod bipolar axon is accompanied at the dyad by a reciprocal amacrine synapse. This differs from the cone bipolar axons, which have reciprocal synapses at some of their dyads but in far lower proportion (2–20%; McGuire et al., '84). The very structure of the reciprocal arrangement implies feedback and the question arises: is it positive or negative? The present results on transmitter accumulation at the dyad in conjunction with Nelson's recording of the responses of the pre- and postsynaptic cells offers a tentative answer.

Most of the rod bipolar's reciprocal amacrine varicosities were found to accumulate 3H-GABA specifically. This probably indicates that these amacrines are genuinely GABAergic because in other mammalian retinas most cells that accumulate GABA are also immunoreactive for endogenous GABA and for its synthetic enzyme glutamic acid decarboxylase (Mosinger et al., '84). The soma of one amacrine type reciprocal to the rod bipolar, A8, has been reported to accumulate glycine (Pourcho and Goebel, '85), but we found glycine accumulation at the dyad only in the AII amacrine cell and not in reciprocal varicosities. We cannot explain this discrepancy at present, except to suggest that the A8 might contribute to only a few (10% or less) of the dyads.

Fig. 7. Functional architecture of the rod bipolar dyad. A: Rod bipolar (RB) is presynaptic to the AII and A17 amacrine. The A17 is in turn presynaptic to the rod bipolar, thus forming a feedback loop. B: Responses of the three neuron types (reprinted with permission from Kolb and Nelson, '83) explained by the functional architecture. The rod bipolar cell hyperpolarizes to a square pulse of light, curtailing its release of inhibitory transmitter. This depolarizes the AII amacrine and A17 amacrine cells which increases the rod bipolar cell's release of inhibitory transmitter. This further hyperpolarizes the rod bipolar, producing further depolarization of both amacrine cells.
Nelson's recordings indicate that the cat rod bipolar cell hyperpolarizes in response to light, curtailing its release of transmitter at the dyad. The AII and A17 amacrine cells, postsynaptic at the dyad, both depolarize to light, suggesting that the rod bipolar transmitter is inhibitory (Nelson, '82; Nelson and Kolb, '85). The fact that noise is reduced in the AII during the light response is additional evidence for a cessation of transmitter release by the rod bipolar cell to the same stimulus and further evidence that in cat the rod bipolar is hyperpolarizing (Nelson, '82). The depolarization of the reciprocal varicosity during the light stimulus would increase its release of GABA onto the rod bipolar axon. Assuming that the GABA effect is mediated by ions with an equilibrium potential negative to the dark potential of the rod bipolar (Miller et al., '81), this would further curtail its release of transmitter. Thus, feedback at the dyad would be positive (Fig. 7).²

The potential uses of negative feedback from reciprocal synapses has been discussed by other investigators: negative feedback can reduce noise, increase response at high frequencies, and, if a suitable time delay between input and output is introduced, it can differentiate the input, leading to a heightened response to change in the input (Koch et al., '86; Dacheux and Raviola, '86). Negative feedback reduces gain, however, an effect which may be unwanted in a pathway devoted to conveying quantal signals.

Positive feedback can also reduce noise and at the same time increase gain. This is because an individual synapse has a certain intrinsic ability to reject noise owing to the exponential or sigmoidal form of its input-output function: in effect there is a presynaptic voltage threshold for synaptic transmission (Katz and Miledi, '67; Martin and Rind, '75; Graubard, '78, Wojtowicz and Atwood, '84; Rind, '84). Assuming that the amplitude of the noise is matched to the low-gain (subthreshold) regions of this function and the signal to the high-gain region, noise in the presynaptic cell would cause little change in the postsynaptic cell's voltage. In a positive feedback loop, such as described for the A17-rod bipolar synapse, the difference in gain between noise and signal would be accentuated because the gain of the loop depends on the product of the gains of the two amplifying elements comprising the loop (Diefenderfer, '72). This multiplicative effect of positive feedback would also increase the total gain of the dyad and lead to a quickening of the rod signal, i.e., a shorter time to reach a given voltage.

The hypothesis of GABA-mediated, positive feedback could be tested by applying bicuculline to a retina adapted to very low background levels of light. This should block the feedback, causing changes in the rod bipolar, AII, and reciprocal amacrine responses. If the feedback is positive and operates as we describe, a reduction in the signal/noise ratio should occur, and also a reduction in gain. If the feedback is negative, a reduction in the signal/noise ratio should occur accompanied by an increase in gain.

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LITERATURE CITED


1Dacheux and Raviola ('86) have found rod bipolar cells in rabbit that depolarize to light and suggested that in cat there might be more than one type of rod bipolar cell, including one that depolarizes. We believe that in cat there is only one morphological type of bipolar with direct input from rods. The hyperpolarizing response reported by Nelson et al. ('76) was attributed to a neuron with a morphology nearly identical with rod bipolar identified in Golgi studies (Boycott and Kolb, '73) and in all our reconstructions (McGuire et al., '84; present paper; Sterling et al., '87b). Although Nelson's recording ultimately prove to be in error, it will more likely be due to an incorrect match between the recording and the stained cell than to the existence of more than one morphological type.

2A possible objection to the hypothesis of positive feedback at the dyad is that the somatic responses of amacrine cells A6, A8, and A13 are, unlike the A17, all hyperpolarizing to light. If their feedback synapses at the dyad were numerous and also hyperpolarizing they would tend to supply negative feedback. However, these cell types all receive numerous inputs from sources other than the rod bipolar cell, while the A17 receives predominant rod bipolar input (Kolb and Nelson, '83; Nelson and Kolb, '85). It is possible, therefore, that the hyperpolarizing responses at the somas of the A6, A8, and A13 reflect these other inputs and that their varicosities at the rod bipolar dyad might be electrically isolated (Ellias and Stevens, '80). Thus at the dyad, their response, like that of the A17, might actually be depolarizing. That the A8 might release glycine does not alter the present argument since glycine, like GABA, has a negative equilibrium potential (Miller et al., '81)
ROD BIPOLAR MICROCIRCUITRY


