A Combined Golgi and Autoradiographic Study of ^3H -Glycine-Accumulating Cone Bipolar Cells in the Cat Retina

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Morphological subclasses of cone bipolar cells in the cat retina were studied in Golgi preparations and through the combination of Golgi impregnation with quantitative electron-microscopic autoradiography of ^3H -glycine accumulation. Camera lucida drawings, computer-assisted rotations, and radial sections provided the morphological details used in assigning individual cells to morphologically defined subclasses. Selected Golgi-impregnated cells from retinas preloaded with ^3H -glycine were sectioned for autoradiographic analysis. Grain densities were quantitated and compared on a normalized scale with the labeling of amacrine cells with ^3H -glycine.

Eleven types of cone bipolar cells were identified, 6 with axon terminals ramifying in sublamina *a* and 5 with axons terminating in sublamina *b*. Of these cells, types CBb2 and CBb5 showed the highest affinity for glycine, with labeling at 41 and 38% with respect to the most heavily labeled amacrine cells. Types CBb1, CBb3, CBa2, and CBa6 were labeled at approximately 20% of maximal labeling. The accumulation of glycine by these cells suggests that they may be involved in providing inhibitory input to the inner retina.

Cone bipolar cells in cat retina play an essential role in the transmission of information from both rod and cone photoreceptor cells to the inner retina (Nelson et al., 1981; Nelson and Kolb, 1983). It is now known that there are a number of morphological subclasses of cone bipolar cells that contribute to the input to ganglion cells. Many of these cells have been described from Golgi studies (Famiglietti, 1981; Kolb et al., 1981), and the physiological responses of some of the cells have been recorded (Nelson and Kolb, 1983). McGuire et al. (1984) reported that there are quantitative differences in the synaptic connections of the different types of cone bipolars, providing further evidence that each morphological subclass comprises a unique neuronal population.

The neurotransmitters mediating cone bipolar transmission have not yet been determined. Physiological studies from carp and mudpuppy retinas have suggested that all bipolar cells are excitatory (Naka, 1976; Dacheux et al., 1979; Werblin, 1979). However, glycine, an inhibitory neurotransmitter, is known to be accumulated by some of the cone bipolar cells in mudpuppy

(Pourcho et al., 1984) and in a variety of mammalian retinas, including cat (McGuire et al., 1980; Pourcho, 1980; Cohen and Sterling, 1986; Pourcho and Goebel, 1986), rabbit (R. G. Pourcho, unpublished observations), and man (Frederick et al., 1984; Marc, 1985; Marc and Liu, 1985).

The present study, undertaken to obtain information about the specific identity of the cone bipolar cells in cat retina that accumulate glycine, involves a combination of Golgi impregnation with electron-microscopic autoradiography. Application of these techniques to the study of glycine accumulation by amacrine cells in the cat retina led to the identification of 4 types of cells that label with ^3H -glycine (Pourcho and Goebel, 1985a). These cells were identified as types A3, A4, A7 (AII), and A8 according to the morphological classification of Kolb et al. (1981). Of these cells, type A8 was the most heavily labeled of all the neurons in the retina. Type A4 amacrine cells were moderately labeled, while A3 and A7 (AII) amacrine cells were lightly labeled.

Prior to initiating the combined Golgi and autoradiographic study of cone bipolar cells, we catalogued a number of these neurons using conventional Golgi techniques to define the basis for assigning individual neurons to specific morphological subclasses. These cells were analyzed through the use of camera lucida drawings, computer rotations, and radial sections. Morphological features were compared with the descriptions provided in the existing nomenclatures for cone bipolar cells (Famiglietti, 1981; Kolb et al., 1981; McGuire et al., 1984). Selected neurons from ^3H -glycine-labeled Golgi preparations were then sectioned for electron-microscopic autoradiography and the relative accumulation of ^3H -glycine by each cell was determined. Two types of cone bipolar cell were found to be moderately labeled, 4 were lightly labeled, and 4 other types appeared essentially unlabeled. As with glycine-accumulating amacrine cells, the relative affinity of each cell type for ^3H -glycine appeared to be a highly consistent feature of that subpopulation.

Materials and Methods

Golgi studies. Adult cat retinas were processed for Golgi impregnation using the method of Mariani in Kolb et al. (1981) and the tissue was flat-embedded in Epon Araldite. A total of 55 cone bipolar cells from 6 particularly well-impregnated retinas was used in the present study. All of the samples were taken from relatively central retina, not more than 5 mm from the optic nerve head. Each cell was photographed and drawn with the aid of a camera lucida using a 100 $\times$ oil-immersion objective. Measurements of somal diameters and of dendritic and axonal field sizes were made from the drawings. The level of stratification of the axon within the inner plexiform layer (IPL) was determined by recording the fine focus calibration at which the axon was in optimal focus and the focal planes for the outer and inner borders of the IPL. From 10 to 30 sets of readings were taken for each cell, and the stratification was calculated as a percentage of the depth of the IPL. These

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Figure 1. Camera lucida drawings of cone bipolar cells whose axons ramify in sublamina *a*. In each case, the dendrite is illustrated in the upper drawing, with the clear soma and the axon terminal in the lower drawing with a shaded soma.

percentages were expressed directly or were converted into stratum assignments within the IPL based upon sublamina *a* as the outer one-third of the IPL (Famiglietti and Kolb, 1975) being equally subdivided into stratum 1 and stratum 2 and sublamina *b*, the inner two-thirds of

the IPL, being divided in 3 equal parts for strata 3–5. Thus, percentage depths of 0–16% were assigned to stratum 1, 17–33% to stratum 2, 34–56% to stratum 3, 57–78% to stratum 4, and 79–100% to stratum 5.

Some of the Golgi-impregnated cells were logged for computer ro-

Figure 2. Camera lucida drawings of cone bipolar cells whose axons ramify in sublamina *b*. As in Figure 1, the *upper drawing* with the *clear soma* illustrates the dendrite, and the *lower drawing* with the *shaded soma* the axon.

tation and then rotated 90°. These rotations provided additional information regarding the branching level of axons with the IPL and helped to correlate the flat-mount appearance of a cell with its appearance in radial section.

Combined Golgi and autoradiography. Adult cats were anesthetized with ketamine (45 mg/kg, i.m.) and sodium pentobarbital (13 mg/kg, i.p.) and given intravitreal injections of 100 μ Ci 3 H-glycine (specific activity, 12 Ci/mmol) per eye. After 4 hr under normal room lighting, these animals were given an overdose of pentobarbital and the retinas were removed for Golgi impregnation. The cells included in the autoradiographic portion of the present study were taken from 3 retinas that were well impregnated with Golgi salts and showed good uptake of 3 H-glycine. After completing morphological examination as described above, selected neurons from these retinas were reembedded and sectioned for electron-microscopic autoradiography. Serial thin sections were made through the somas of identified cone bipolar cells and allowed to dry down on collodion-coated slides. The sections were then covered with a Formvar film and dipped into Ilford L-4 emulsion (Goebel and Pourcho, 1982). The Formvar covering prevents chemographic exposure of the emulsion by the Golgi salts. The autoradiographs were developed after a 3 week exposure.

The degree of labeling of individual cells was determined from electron-microscopic autoradiographs of a series of 12–16 successive thin sections. Grain counts were made from at least 10 neurons in each series, including the Golgi-impregnated cell and the heaviest labeled cell. Areas for each cell were determined using a Bio-Quant program and label densities calculated in grains/ μ m². Grain densities were corrected for background and then normalized against a value of 1.0 for the cell with the highest grain density. These methods have been employed previously in studies of amacrine cells (Pourcho and Goebel, 1983, 1985a).

Results

Morphology

Cone bipolar cells from cat retinas were classified according to the morphological appearance and branching patterns of their dendrites and axons; lamination of the axons within the IPL; size and location in the inner nuclear layer (INL) of somas; and dendritic and axonal field diameters. Camera lucida drawings (Figs. 1, 2), computer rotations (Figs. 3, 4), and measurements of size and laminar distribution were made for each cell type. Tables 1 and 2 summarize this data. Each cell was evaluated in relation to the 3 existing systems of nomenclature for cone bipolar cells in the cat retina (Famiglietti, 1981; Kolb et al., 1981; McGuire et al., 1984). In the present paper, we have chosen to pattern our nomenclature on that put forth by McGuire et al. (1984) in the most recent of these publications. Thus, we have used the name CBa1 to designate the cone bipolar cell that ramifies in sublamina *a* with morphological features resembling the CBa₁ of McGuire et al. (1984). Efforts were made to relate each cell to its appropriate counterpart in the classification systems of Kolb et al. (1981) and Famiglietti (1981).

CBa1 cone bipolar cells have somas located near the middle of the INL. A single dendrite arises from the soma and ascends to the outer plexiform layer (OPL), where it branches into 3–5 secondary dendrites of medium thickness (Fig. 1). After one or more additional branchings, these dendrites give rise to 1–2

Figure 3. Computer-assisted rotations of cone bipolar cells ramifying in sublamina *a*.

Figure 4. Computer-assisted rotations of cone bipolar cells ramifying in sublamina *b*.

terminal digits. The axon is smooth and ramifies in the outer third of the IPL, entirely within sublamina *a* (Fig. 3). Dendritic field diameters varied from 30 to 45 μm , with axonal spreads of approximately the same range (Table 1).

On the basis of their soma position in the mid-INL, smooth axonal contours, and ramification in S1–2, CBa1 cells were equated with the CBa₁ cells of McGuire et al. (1984). The narrow stratification of this cell in sublamina *a* (Fig. 2) indicates a correspondence with type cb2 of Kolb et al. (1981), which makes flat synaptic contacts with cone pedicles (Boycott and Kolb, 1973; Nelson and Kolb, 1983; McGuire et al., 1984). The moderately thick dendrites resemble those exhibited by the na1 of Famiglietti (1981).

CBa2 cells were distinguished by their thick dendrites and large axonal varicosities (Fig. 1). The dendrites often appear twisted and give rise to clusters of 2–3 terminal digits. Most of the cell bodies were located in the middle layers of the INL. Somas were large, measuring up to 11 μm in diameter. Dendritic fields were slightly smaller than those of CBa1 cells and ranged

from 25 to 40 μm . In most cases, the axonal field of a given cell was larger than its dendritic spread (Table 1).

The prominent axonal varicosities (Fig. 1) of CBa2 cells resemble those of CBa₂ (McGuire et al., 1984) and cb1 (Kolb et al., 1981) cells. The thick dendrites correspond with those of na2 cells of Famiglietti (1981), while the clustered terminal digits were described for both cb1 (Kolb et al., 1981) and na2 (Famiglietti, 1981). These terminal digits appear well suited for receiving ribbon-related invaginating contacts from cones as described by McGuire et al. (1984).

CBa3 cells were identified primarily by their dendritic terminals, which appeared to surround individual cone pedicles in a clamplike configuration (Fig. 1). Although not every dendrite on a particular cell terminated in this manner, 4 or more “clamps” were seen on each cell, and 1 cell exhibited at least 10. The dendrites were of moderate thickness and arose as 1 or 2 stalks from the cell body, which was found in the middle layers of the INL. Soma sizes varied but averaged 9–10 μm . Axons were finely varicose and ramified primarily within S1

Table 1. Cone bipolar cells of sublamina α : Cell characteristics and ^3H -glycine accumulation

Cell type	Grain density (gr/ μm^2)	Soma position in INL	Soma diameter (μm)	Axon lamination (%)	Dendritic field size (μm)	Axonal field size (μm)
CBa1						
Cell 1	0.12	49	10.5	16–33	54 × 40	32 × 30
Cell 2	0.08	50	9.7	5–31	38 × 17	46 × 29
Cell 3	NS	44	10.0	8–17	46 × 41	54 × 22
Cell 4	NS	56	9.5	4–27	38 × 24	35 × 27
Cell 5 ^{a,b}	NS	59	7.9	10–30	34 × 24	43 × 34
Average	0.10				42 × 29	42 × 28
CBa2						
Cell 6	0.19	50	11.6	4–16	44 × 25	48 × 40
Cell 7	0.18	36	10.5	10–29	39 × 23	45 × 28
Cell 8	0.19	35	11.1	15–25	25 × 16	42 × 25
Cell 9	NS	45	11.1	9–22	55 × 38	49 × 23
Cell 10 ^{a,b}	NS	47	11.0	4–25	36 × 17	58 × 30
Cell 11	NS	65	11.1	15–25	33 × 20	49 × 33
Cell 12	NS	71	7.4	10–24	27 × 22	27 × 15
Cell 13	NS	72	9.0	17–30	28 × 28	37 × 34
Cell 14	NS	76	9.5	11–25	34 × 38	41 × 35
Cell 15	NS	63	10.0	13–25	23 × 18	42 × 18
Cell 16	NS	65	10.5	—	50 × 38	35 × 30
Cell 17	NS	82	8.7	4–30	32 × 25	52 × 23
Average	0.19				36 × 21	44 × 28
CBa3						
Cell 18 ^b	0.11	35	9.5	16–31	41 × 28	46 × 23
Cell 19	0.03	50	9.5	20–30	36 × 31	38 × 32
Cell 20 ^a	0.07	60	10.0	11–32	50 × 19	42 × 34
Cell 21	0.13	50	7.9	12–30	37 × 21	37 × 20
Cell 22	NS	73	10.0	15–41	48 × 24	33 × 18
Cell 23	NS	30	9.9	13–29	29 × 24	30 × 22
Cell 24	NS	30	9.5	15–27	52 × 37	57 × 33
Cell 25	NS	64	10.0	12–29	39 × 18	42 × 20
Cell 26	NS	44	12.6	13–44	52 × 26	53 × 23
Average	0.08				43 × 25	42 × 25
CBa4						
Cell 27 ^{a,b}	0.09	57	9.2	21–41	54 × 26	50 × 29
Cell 28	0.13	60	9.2	25–46	36 × 19	35 × 30
Cell 29	NS	72	9.0	17–22	52 × 34	40 × 39
Cell 30	NS	39	9.2	30–40	47 × 35	46 × 30
Cell 31	NS	81	8.7	30–40	—	51 × 37
Cell 32	NS	57	9.5	15–25	36 × 18	38 × 13
Average	0.11				56 × 26	52 × 36
CBa5						
Cell 33 ^{a,b}	NS	70	9.5	15–23	72 × 54	92 × 45
CBa6						
Cell 34 ^{a,b}	0.19	5	15.0	18–28	106 × 78	57 × 43

NS, Cell used for morphology only, and not sectioned for autoradiography.

^a This cell is illustrated in the camera lucida drawing in Figure 1.^b This cell is illustrated in the computer rotation in Figure 3.

and S2, although in some cases the terminal processes continued into S3 (Fig. 3). Dendritic and axonal fields were often oval, with a nearly 2-fold difference in length versus width (Table 1).

The dendrites of CBa3 cells resemble those of type na3 cells of Famiglietti, which were described as forming "ring-like garlands at the bases of cone pedicles" (Famiglietti, 1981). These cells also correspond to CBa₃ of McGuire et al. (1984), which

exhibits initial axonal branching in S1 and outer S2 (Fig. 2). As noted by McGuire et al. (1984), the axonal contours of these cells are similar to those of CBa1. Their correspondence in the classification of Kolb et al. (1981) is with type cb2 or a subset of cb2.

CBa4 cells were recognized by their thin, sparsely branching dendrites (Fig. 1). The axons are somewhat varicose and often

Table 2. Cone bipolar cells of sublamina *b*: Cell characteristics and ³H-glycine accumulation

Cell type	Grain density (gr/μm ²)	Soma position in INL (%)	Soma diameter (μm)	Axon lamination (%)	Dendritic field size (μm)	Axonal field size (μm)
CBb1						
Cell 35 ^{a,b}	0.26	15	11.0	61–84	34 × 14	30 × 15
Cell 36	0.19	44	11.6	60–80	30 × 23	31 × 27
Cell 37	NS	—	8.9	50–82	25 × 20	20 × 18
Cell 38	NS	83	9.2	52–78	30 × 27	41 × 17
Cell 39	NS	26	8.2	44–59	33 × 13	32 × 21
Cell 40	NS	—	9.5	43–61	22 × 18	28 × 25
Cell 41	NS	80	11.6	53–82	26 × 19	20 × 15
Average	0.22				33 × 19	29 × 20
CBb2						
Cell 42 ^{a,b}	0.35	63	7.4	43–84	28 × 23	29 × 27
Cell 43	0.47	90	7.6	40–74	34 × 26	42 × 23
Cell 44	NS	—	7.4	39–50	23 × 21	29 × 19
Cell 45	NS	66	7.9	50–67	37 × 19	36 × 31
Average	0.41				31 × 22	34 × 25
CBb3						
Cell 46	0.22	20	11.1	1–10 53–86	36 × 47	17 × 6 40 × 34
Cell 47	NS	5	11.6	3–15 55–78	45 × 18	21 × 16 38 × 28
Cell 48 ^{a,b}	0.25	50	10.5	3–17 45–65	35 × 25	13 × 10 34 × 27
Average	0.23				39 × 30	17 × 11 37 × 30
CBb4						
Cell 49 ^b	0.12	95	8.9	32–51	22 × 25	29 × 27
Cell 50	NS	90	8.0	36–52	34 × 20	28 × 27
Cell 51	NS	71	4.5	29–46	33 × 16	38 × 26
Cell 52	NS	90	8.9	28–56	17 × 10	30 × 29
Cell 53 ^a	NS	90	8.9	36–56	23 × 28	31 × 25
Average					26 × 20	31 × 27
CBb5						
Cell 54 ^{a,b}	0.38	50	9.5	55–88	89 × 58	51 × 35
Cell 55	NS	25	12.6	46–68	110 × 100	62 × 62
Average					100 × 79	57 × 49

NS, Cell used for morphology only and not sectioned for autoradiography.

^a This cell is illustrated in the camera lucida drawing in Figure 2.^b This cell is illustrated in the computer rotation in Figure 4.

exhibit spines. Soma sizes were consistently 9 μm in diameter. The cell bodies are usually located in the lower half of the INL and the dendrites derive from 1 or 2 ascending processes. Axons ramify narrowly near the *a/b* sublaminal border at a 30–40% depth level in the IPL (Fig. 3). Dendritic spreads measured 30–40 μm, while axonal spreads were similar. These cells correspond to CBa₄ of McGuire et al. (1984), cb3 of Kolb et al. (1981), and type nab of Famiglietti (1981).

CBa5 cells have larger dendritic and axonal spreads, each measuring approximately 60 μm across (Fig. 1). These cells were relatively rare in our samples. The dendrite is thick and usually terminates in single digits, while the axon is smooth and spreading. It ramifies near the middle of S2 (Fig. 3).

CBa6 cells have the widest dendritic fields and may cover 100 μm of the OPL (Fig. 1). The very large (14 μm) cell body is located in the outer portion of the INL and gives rise to 2 or

3 thick dendrites that course in a tortuous path through the OPL with little branching. The axon is smoothly varicose and ramifies in S2. The diameter of the axonal field is about half that of the dendritic spread (Table 1).

The cells designated CBa5 and CBa6 do not have any correlates in the nomenclature of McGuire et al. (1984). The larger field size and smooth axon of the CBa5 resemble the medium field cone bipolar (ma) of Famiglietti (1981). CBa6 cells are the wide-field cells of sublamina *a* and correspond to cb7 of Kolb et al. (1981) and wa of Famiglietti (1981).

CBb1 cells are the sublamina *b* counterpart of CBa2, having thick dendrites with 2–3 terminal digits and prominent axonal varicosities (Fig. 2). The varicosities tend to be vertically oriented. The axon may give rise to terminal processes that descend through S3–5 or may continue, unbranched, into S4 or 5 and give rise to terminal processes that turn upward in a “fishhook”

Figure 5. Typical electron-microscopic autoradiograph showing a Golgi-impregnated cone bipolar cell that has accumulated ^3H -glycine. A number of other cells (1–9) in the inner nuclear layer (INL) are also labeled. Grain densities were calculated as the average number of silver grains per unit area for these cells and normalized with respect to the most heavily labeled cell. Arrows show processes of the Golgi-impregnated cell extending into the inner plexiform layer (IPL). Bar, 1 μm .

configuration. Cell bodies measured from 8 to 11 μm in diameter and were found at any level in the INL. Both dendritic and axonal fields were 20–30 μm across (Table 2). CBb1 cells correspond to CBb₁ of McGuire et al. (1984), cb5 of Kolb et al. (1981), and nb2 of Famiglietti (1981).

CBb2 cells resemble CBa1 cells and have moderately thick dendrites with 1–2 terminal digits (Fig. 2). The axons appear scalloped and extend horizontally in S3 and S4. Cell bodies were the smallest of the cone bipolars and measured less than 8 μm in diameter. They were located in the inner half of the INL. Both dendritic and axonal spreads were narrow, ranging from 25 to 35 μm across. The CBb2 cells correspond to CBb₂ of McGuire et al. (1984), cb6 of Kolb et al. (1981), and nb1 of Famiglietti (1981).

Type CBb3 cells are bistratified cone bipolar cells with varicose terminals in sublamina *b* and a smaller, fine ramification in sublamina *a*. The somas of these cells measure 10–11 μm in diameter and are located high in the INL, where they give rise to 2–3 extremely thick dendrites and several smaller ones. The large dendrites terminate in clusters of 3–4 digits, while the smaller ones are unbranched (Fig. 2). Both dendritic and axonal fields are 35–45 μm in diameter. The major axonal process is varicose and ramifies in S3–4. Many of these processes appear

to be vertically oriented, resembling the CBb1. However, the CBb3 cells give rise to 1 or more thin processes that extend in the outer part of S1, just deep to the amacrine cell layer, resulting in a bistratified appearance as seen in the computer rotation of this cell (Fig. 4). Type CBb3 cells are further distinguished from CBb1 cells by their thicker dendrites and relatively few terminal digits (Fig. 2). They correspond to CBb₃ cells of McGuire et al. (1984) and may also be equivalent to cb4 of Kolb et al. (1981), which appears more highly developed in peripheral retina.

CBb4 cells have somas in the amacrine layer and measure 8–9 μm in diameter. These neurons give rise to a single wispy dendrite that extends up to the OPL, where it divides into several relatively unbranching processes (Fig. 2). The axon descends to S3, where it gives rise to a finely varicose, bushy arborization. Dendritic and axonal field diameters range from 20 to 30 μm . The clumpy appearance of the axon is also distinctive (Fig. 2). These neurons resemble the CBa4 cells in that they ramify at or near the *a/b* sublamina border. However, they are found primarily within S3 and have much thinner dendrites than the CBa4 cells (Figs. 1, 3). There is no clear correlate of this cell in the study of McGuire et al. (1984). However, CBa4 is likely a subset of the nab cells seen by Famiglietti (1981) and the cb3 of Kolb et al. (1981).

Autoradiography

Cell type	McGuire et al. (1984)	Kolb et al. (1981)	Famigli- etti (1981)
CBa1	CBa ₁	cb2	na1
CBa2	CBa ₂	cb1	na2
CBa3	CBa ₃	cb2	na3
CBa4	CBa ₄	cb3	nab
CBa5	—	—	ma
CBa6	—	cb7	wa
CBb1	CBb ₁	cb5	nb2
CBb2	CBb ₂	cb6	nb1
CBb3	CBb ₃	cb4	—
CBb4	—	cb3	nab
CBb5	—	cb8	wb

Discussion

Autoradiography of glycine accumulation by cone bipolar cells, like that of amacrine cells (Pourcho and Goebel, 1985a), showed a remarkable degree of consistency among different examples of a single cell type (see Tables 1 and 2). Thus, transmitter content, like morphological characteristics and synaptic connectivity, appears to be a highly specific property of each subpopulation. The present data were derived from animals maintained during normal room lighting and the possibility of significant differences in glycine levels under different stimulus conditions remains to be investigated.

A recurring concern in autoradiographic studies is that high-affinity uptake may not accurately reflect endogenous levels of

the material in question. To address this question, a parallel immunocytochemical study was carried out using an antiserum generated against glycine (Pourcho and Goebel, 1985b, 1986b). Distribution of the immunoreactivity appears to confirm the localizations seen by use of autoradiography (Goebel and Pourcho, 1986), indicating that the values reported here reflect endogenous as well as accumulated glycine. Staining was observed in at least 4 classes of amacrine cell, including the A7 (AII), in cone bipolar cells terminating in sublamina *a*, and in other cone bipolars (CBb1 and CBb2) terminating in sublamina *b* (Pourcho and Goebel, 1987).

Physiological studies have consistently demonstrated that glycine is a powerful inhibitor in the cat retina, producing selective effects on certain ganglion cells and reducing the light-evoked response of all ganglion cells (Kirby, 1979; Ikeda and Shearman, 1983; Bolz et al., 1985). While many of these effects are thought to be due to glycinergic amacrine cells, the role of glycine in bipolar cells remains a matter of much speculation. Sterling and McGuire (Sterling, 1983; McGuire et al., 1984, 1986) have proposed a "push-pull" hypothesis in which both excitatory and inhibitory cone bipolar cells provide input to beta ganglion cells. They have provided morphological support for this hypothesis by reconstructing the synaptic input to these cells (McGuire et al., 1986). At least 2 different types of cone bipolar were found to synapse on both On- and Off-beta cells. In the case of On-ganglion cells, the inputs were identified as being from CBb₁ and CBb₂ cone bipolars, while Off-cells received input from CBa₁ and CBa₂ cells. The authors theorize that CBa₁ and CBb₁ are excitatory, while CBa₂ and CBb₂ are inhibitory.

Wässle et al. (1986) have recently utilized the On-pathway blocker, aminophosphonobutyric acid (APB) to explore glycine inhibition in the cat retina. Their findings are consistent with the "push-pull" model in which hyperpolarizing cone bipolars terminating on On-center ganglion cells and depolarizing bipolar cells to Off-center ganglion cells utilize glycine as a transmitter. Types CBb₂ and CBa₂ cone bipolar cells appear to fulfill these criteria. CBb₂ cells are hyperpolarizing and synapse upon On-ganglion cells (Nelson and Kolb, 1983). CBa₂ cells have not been recorded from but are known to synapse on Off-ganglion cells (Kolb, 1979; McGuire et al., 1980, 1984, 1986) and are hypothesized to be depolarizing. In the present study, types CBb₂ and CBa₂ were found to be the most heavily labeled cone bipolar cells in their respective sublaminae, consistent with the predictions of the model.

Other cells that were labeled with glycine showing grain densities equal to or greater than that of CBa₂ cells included CBa₆, CBb₁, CBb₃, and CBb₅. There is very little information about CBa₆, CBb₃, and CBb₅ cells. Type CBb₁ cells have been studied extensively and found to make extensive gap junction contacts with A7 (AII) amacrine cells (Kolb and Famiglietti, 1974; McGuire et al., 1984). Cohen and Sterling (1986) also reported an accumulation of ³H-glycine by CBb₁ cells. Since these cells are postulated to be excitatory in the "push-pull" model, these authors suggest that the accumulation of glycine may result from diffusion through the gap junctions with A7 (AII) amacrine cells, which have been shown to accumulate glycine (Pourcho, 1980; Pourcho and Goebel, 1985a). If spillover occurs, its significance and the mechanism by which it is controlled remain to be elucidated. A possible candidate for modulation of such leakage is dopamine. Vaney (1985) reports preliminary evidence that the dopamine antagonist haloperidol increases dye coupling between AII amacrine cells and other neurons of the inner nuclear

layer. In support of this possibility, there is strong evidence that dopamine participates in the control of gap junctions between horizontal cells in the outer plexiform layer (Negishi and Drujan, 1979; Cohen and Dowling, 1983). However, as suggested by Wässle et al. (1986), it is unlikely that transmitter spillover would occur between cells using different transmitters. The present finding that CBb₂ cells accumulate ³H-glycine more avidly than A7 (AII) amacrine cells with which they have been reported to make gap junction contacts (McGuire et al., 1984) is indicative of a preferential uptake by these cone bipolars rather than a passive diffusion from the amacrine cells.

The cells found to be labeled with ³H-glycine in the present study include subpopulations of cone bipolars whose axons ramify in sublamina *a* as well as subpopulations ramifying in sublamina *b*. Cohen and Sterling (1986) recently reported labeling of cone bipolars in sublamina *b* only. Since their data does not specifically include CBa₂ or CBa₆ cells, it is difficult to make direct comparisons of the results. Differences in the 2 studies may also derive from our use of longer time intervals following intravitreal injection of ³H-glycine. The 4 hr interval used in our experiments was based on evidence from mouse retina that labeled amino acids from an intravitreal injection may require several hours to reach equilibrium levels throughout the retina (Pourcho, 1977).

In summary, this study has provided a review of the Golgi morphology of cone bipolar cells in the cat retina and furnished a quantitative assessment of glycine accumulation by these cells. Camera lucida drawings, computer-assisted rotations, and radial sections provided a basis for describing the unique morphology, branching pattern, and lamination of each cell type. Electron-microscopic autoradiography showed the accumulation of ³H-glycine by 6 of the cone bipolar classes, with types CBb₂ and CBb₅ exhibiting the highest grain densities of any of the bipolars. While identification of CBa₂ and CBb₂ cells as the most heavily labeled cone bipolars in their respective sublaminae is consistent with their postulated roles in the "push-pull" model, the remaining subclasses must also be considered as possible contributors of glycinergic input to the inner retina.

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