THE JOURNAL OF COMPARATIVE NEUROLOGY 421:1–13 (2000)

The Dendritic Architecture of the Cholinergic Plexus in the Rabbit Retina: Selective Labeling by Glycine Accumulation in the Presence of Sarcosine

DAVID I. VANEY* AND DAVID V. POW

Vision, Touch and Hearing Research Centre, Department of Physiology and Pharmacology, The University of Queensland, Brisbane, Queensland, Australia

ABSTRACT

The cholinergic amacrine cells in the rabbit retina slowly accumulate glycine to very high levels when the tissue is incubated with excess sarcosine (methylglycine), even though these cells do not normally contain elevated levels of glycine and do not express high-affinity glycine transporters. Because the sarcosine also depletes the endogenous glycine in the glycine-containing amacrine cells and bipolar cells, the cholinergic amacrine cells can be selectively labeled by glycine immunocytochemistry under these conditions. Incubation experiments indicated that the effect of sarcosine on the cholinergic amacrine cells is indirect: sarcosine raises the extracellular concentration of glycine by blocking its re-uptake by the glycinergic amacrine cells, and the excess glycine is probably taken-up by an unidentified low-affinity transporter on the cholinergic amacrine cells. Neurobiotin injection of the On-Off direction-selective (DS) ganglion cells in sarcosine-incubated rabbit retina was combined with glycine immunocytochemistry to examine the dendritic relationships between the DS ganglion cells and the cholinergic amacrine cells. These double-labeled preparations showed that the dendrites of the DS ganglion cells closely follow the fasciculated dendrites of the cholinergic amacrine cells. Each ganglion cell dendrite located within the cholinergic strata is associated with a cholinergic fascicle and, conversely, there are few cholinergic fascicles that do not contain at least one dendrite from an On-Off DS cell. It is not known how the dendritic co-fasciculation develops, but the cholinergic dendritic plexus may provide the initial scaffold, because the dendrites of the On-Off DS cells commonly run along the outside of the cholinergic fascicles. J. Comp. Neurol. 421:1–13, 2000.

Indexing terms: cholinergic amacrine cells; glycine uptake; methylglycine; direction-selective ganglion cells; dendritic fasciculation; Neurobiotin

Although the cholinergic amacrine cells in the rabbit retina are one of the best characterized types of amacrine cells in any retina, new findings about these cells have continued to surprise retinal neuroscientists in the 20 years since the cells were first identified by Masland and Mills (1979). Four characteristics in particular stand out as novel and unexpected (Vaney, 1990). First, there are two mirror-symmetric populations of cholinergic amacrine cells, one with somata in the inner nuclear layer and the other with somata displaced to the ganglion cell layer (Hayden et al., 1980; Vaney et al., 1981). The orthotopic cells stratify narrowly within sublamina *a* of the inner plexiform layer whereas the displaced cells stratify narrowly within sublamina *b*. Correspondingly, the type *a* cholinergic (C*a*) cells are Off-center cells, whereas the type *b* cholinergic (C*b*) cells are On-center cells (Bloomfield and

Grant sponsor: National Health and Medical Research Council (Australia).

*Correspondence to: David I. Vaney, Vision, Touch and Hearing Research Centre, The University of Queensland, Brisbane, QLD 4072, Australia. E-mail: vaney@vthrc.uq.edu.au

Received 17 July 1999; Revised 7 October 1999; Accepted 13 October 1999

Miller, 1986). Second, the dendritic-field overlap of the cholinergic amacrine cells is an order of magnitude greater than that of integrating retinal neurons (Tauchi and Masland, 1984; Vaney, 1984). Despite this overlap, the cholinergic dendrites do not blanket the retina but show a striking fasciculated topology, with bundles of dendrites surrounding dendrite-free lacunae (Tauchi and Masland, 1985). Third, the distinctive "starburst" dendritic morphology of the cholinergic amacrine cells reflects the spatial segregation of the synaptic connections, with the excitatory input from bipolar cells distributed over the whole dendritic tree and the synaptic output to ganglion cells confined to the varicose distal zone (Famiglietti, 1991). This input-output offset appears important for the function of cholinergic amacrine cells in facilitating the responses of ganglion cells to image motion (He and Masland, 1997). Fourth, the cholinergic amacrine cells contain, synthesize and accumulate γ-aminobutyric acid (GABA) (Brecha et al., 1988; Vaney and Young, 1988).

We now report that the cholinergic amacrine cells in the rabbit retina can also accumulate glycine, by an uptake mechanism that is distinct from that used by glycinergic amacrine cells. Almost all amacrine cells contain elevated levels of glycine or GABA and, in the rabbit retina, the putative glycinergic neurons account for ≈60% of the amacrine cells (Crook and Pow, 1997; Wright, 1998). The glycine-containing amacrine cells express the glycine transporter GLYT1 (Menger et al., 1998; Vaney et al., 1998; Pow and Hendrickson, 1999) and they can be reversibly depleted of their endogenous glycine by incubation with sarcosine (Pow, 1998), which is a competitive inhibitor of GLYT1 and thus prevents the re-uptake of released glycine (Guastella et al., 1992). In the rabbit retina, however, the cholinergic amacrine cells accumulate glycine to very high levels in the presence of sarcosine, which presumably increases the concentration of extracellular glycine that is accessible to the cholinergic cells. This uptake mechanism enables the cholinergic plexus to be visualized with remarkable clarity in glycine-immunolabeled wholemounts, thus providing a novel tool for studying the dendritic relationships between the cholinergic amacrine cells and other retinal neurons. Parts of this study have been reported in preliminary form (Vaney et al., 1989; Pow and Vaney, 1998).

MATERIALS AND METHODS

All experiments were approved by the University of Queensland animal experimentation ethics committee and were conducted in accord with the Australian code of practice for the care and use of animals for scientific purposes. Adult pigmented rabbits and Dark Agouti rats were obtained from the University of Queensland Central Animal House. Biochemical reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless indicated otherwise.

Sarcosine incubation

Rats were euthanased by intraperitoneal injection of sodium pentobarbital, the eyes enucleated and hemisected, the retina removed and placed in carbogenated serum-free Ames medium at 36°C under normal room light (Ames and Nesbett, 1981). The retinas were incubated with 0.5 mM sarcosine in Ames medium for 3 hours (Pow, 1998). Rabbits were euthanased by intravenous injetion of sodium pentobarbital, the eyes enucleated and hemisected, and the posterior eyecup placed in Ames medium at room temperature or 36°C. The eyecups were usually incubated with 0.5–1.0 mM sarcosine in Ames medium for 4–6 hours. For immunolabeling of retinal wholemounts and Vibratome transverse sections, the tissue was fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 60–90 minutes. For immunolabeling of semithin resin sections, the tissue was fixed with 2.5% glutaraldehyde in phosphate buffer for 16 hours.

Immunocytochemistry

The retinas were immunolabeled for glycine using our well-characterized antisera, according to established protocols that are detailed elsewhere (Pow and Crook, 1993; Pow et al., 1995; Wright et al., 1997). The glycine antisera have previously been demonstrated not to cross-react with a variety of amino acids that occur naturally in the retina. We have subsequently demonstrated that the antisera do not cross-react with sarcosine dot blots that have been exposed to aldehydes (data not shown) and it would be expected that the methylation of the amino group would prevent the fixation of sarcosine with aldehydes. Furthermore, the glycine antisera did not cross-react with sarcosine dot blots that were fixed with carbodiimide, which binds to the carboxyl group.

Autoradiography

To examine the effects of sarcosine on the uptake of exogenous glycine, pieces of rabbit retina attached to the sclera were initially incubated for 30 minutes at room temperature in serum-free Ames medium, which contains 6 μM glycine. The tissue was then incubated for 30–180 minutes in Ames medium containing 1.0 mM sarcosine and 50 μl/ml of ^{3}H-glycine (16.2 Ci/mM; TRK71, Amersham, Little Chalfont, England), giving a final glycine concentration of 9 μM. The tissue was further incubated in Ames medium for 30 minutes before fixation in 4% paraformaldehyde and 0.2% glutaraldehyde in phosphate buffer for 90 minutes at 4°C. The retina was embedded in resin and processed for autoradiography, as described elsewhere (Wright et al., 1997).

DAPI labeling

The rabbits were deeply anaesthetized with 4% halothane, and 0.4 μg of 4,6-diamidino-2-phenylindole (DAPI) in 20 μl of water was injected into the vitreous chamber, 1–2 days prior to the intracellular injection experiments (Masland et al., 1984). DAPI is a fluorescent nuclear dye that selectively labels three types of amacrine cells in the rabbit retina, comprising the C*a* and C*b* cholinergic cells and the glycinergic DAPI-3 cells (Tauchi and Masland, 1984; Vaney, 1984; Wright et al., 1997). The nuclei of retinal ganglion cells are only weakly labeled by DAPI but they are clearly defined and differ in their shape and size. Many of the On-Off direction-selective (DS) ganglion cells have a large crescent-shaped nucleus, enabling these cells to be targeted for intracellular injection with a high success rate (Vaney et al., 1989; Vaney, 1994).

Intracellular dye injection

Our procedures for preparing the superfused retinal wholemount and for intracellular injection of microscopically identified neurons with either Lucifer yellow or Neu-

robiotin have been described in detail elsewhere (Vaney, 1984, 1991; Hampson et al., 1992). In all of the Neurobiotin-injection experiments, the rabbit retina was incubated and superfused with 1.0 mM sarcosine in Ames medium at room temperature. After being immersed in this solution for ≈5 hours, the tissue was fixed for glycine immunolabeling of the wholemount. The Neurobiotin in the injected cells was visualized with FITC-tagged streptavidin (Amersham, Little Chalfont, England) and the accumulated glycine in the cholinergic amacrine cells was visualized with Texas Red-tagged secondary antibodies, according to our published double-labeling protocol (Wright et al., 1997). In one series of experiments, Lucifer yellow was injected into an On-Off DS ganglion cell and overlapping C*b* amacrine cells (Vaney et al., 1989). Following fixation in 4% paraformaldehyde for 60 minutes, the Lucifer yellow was converted to an opaque reaction product by photo-oxidation in the presence of diaminobenzidine (Maranto, 1982), as detailed by Vaney et al. (1991).

Photomicrography

The immunofluorescence preparations (see Figs. 4–6) were imaged with a BioRad MRC 600 confocal laser-scanning microscope (768 × 512 pixels) mounted on a Zeiss Axioskop; FITC and Texas Red were visualized using the blue and green lines, respectively, of the krypton-argon laser. The immunoperoxidase wholemounts (see Fig. 2) and the autoradiograph (see Fig. 3) were imaged with a Hamamatsu video camera (1,000 × 1,000 pixels) mounted on an Olympus microscope. Photomicrographs of the immunoperoxidase sections (see Fig. 1) and the Lucifer-injected cells (see Fig. 7) were taken on various Zeiss microscopes and the images digitized with a Nikon film scanner.

Image processing

The brightness and contrast of the digital images were adjusted using Adobe PhotoShop 5 and most images were sharpened using the Unsharp Mask filter. The confocal micrographs of the double-labeled preparations (see Figs. 5, 6B) were subject to special treatment. Two Z-series of images was taken at 1 μm intervals through the inner plexiform layer of the wholemount to record the morphology of 1) the Neurobiotin-injected cells and 2) the overlapping glycine-immunoreactive cholinergic cells. In each confocal image, the dendrites in sublamina *b* of the inner plexiform layer were selected using the lasso tool and these dendrites were then collapsed down to a single image, thus excluding the dendrites in sublamina *a* and the somata in the ganglion cell layer. The contrast of the glycine-immunoreactive C*b* plexus was inverted and reduced, and the variations command of PhotoShop was used to add red to the midtones and shadows, and to add cyan to the highlights. The contrast of the Neurobiotin-injected cells was inverted and increased, and this black/white image was then multiplied by the red/cyan image of the C*b* dendritic plexus.

RESULTS

Sarcosine has different effects on rat and rabbit retinas

Figure 1 shows transverse sections of rat and rabbit retinas that were incubated for several hours in either Ames medium containing excess sarcosine or control Ames medium. The experimental and control tissues were embedded together in the resin block and they were subject to identical glycine immunolabeling and silver intensification. The control rat retina shows the normal pattern of glycine immunoreactivity, with moderate to strong labeling of many amacrine cells and bipolar cells (Fig. 1A). The elevated levels of endogenous glycine in these cells are almost totally depleted following incubation with 0.5 mM sarcosine for 3 hours (Fig. 1B), and the same effect has been observed in chicken, cat, and monkey retinas (Pow, 1998). The pattern of glycine immunoreactivity in the control rabbit retina is comparable to that in the control rat retina, although the glycine-immunopositive bipolar cells are less pronounced in the rabbit retina (Fig. 1C).

Incubation of rabbit retina with 0.5 mM sarcosine for 4 hours has two contrasting effects (Fig. 1D). As expected, the glycine-containing amacrine and bipolar cells lose much of their glycine, although the depletion is not as complete as found in the rat retina. Much more striking, however, is the development of glycine immunoreactivity in two populations of cells: one type has strongly labeled somata in the inner nuclear layer and stratifies narrowly at ≈20% depth of the inner plexiform layer, whereas the other type has intensely labeled somata in the ganglion cell layer and stratifies narrowly at ≈70% depth. These cells show much stronger glycine-like immunoreactivity than the most intensely labeled glycinergic amacrine cells in the control retina.

It was immediately apparent to us that the mirror-symmetric populations of glycine-immunoreactive cells correspond to the mirror-symmetric populations of cholinergic amacrine cells: their appearance in transverse section matches that obtained with immunolabeling for choline acetyltransferase (ChAT) (Brandon, 1987; Famiglietti and Tumosa, 1987). This identity was further supported by their appearance in retinal wholemounts (Fig. 2C–F). Each population is present at a density of ≈350 cells/mm^2 in mid-peripheral rabbit retina, matching the density of cholinergic amacrine cells at this eccentricity (Vaney et al., 1981). Moreover, the pronounced fasciculation of the cholinergic plexus is readily apparent in the glycine-immunolabeled wholemounts (Tauchi and Masland, 1985). We did not directly test whether the glycine-immunoreactive cells contain ChAT, partly because their identity was so obvious and partly because ChAT immunolabeling is problematic in the rabbit retina. Indeed, the glycine immunolabeling shows the structure of the C*b* dendritic plexus much more clearly than has been possible with either ChAT immunolabeling or neurofibrillar methods.

Four to six hours were required for the sarcosine-induced glycine immunoreactivity to reach maximum contrast. Both the depletion of endogenous glycine from the glycinergic amacrine cells and the accumulation of glycine by the cholinergic amacrine cells developed slowly, regardless of whether the incubation was undertaken at 36°C or room temperature. The C*a* amacrine cells were invariably less glycine immunoreactive than the C*b* amacrine cells, even at the cut edge of retinas where the penetration of sarcosine or antibodies would not be a limiting factor. In some preparations, the levels of accumulated glycine in the C*a* somata were only slightly greater than the levels of depleted glycine in the glycinergic amacrine cells. In other

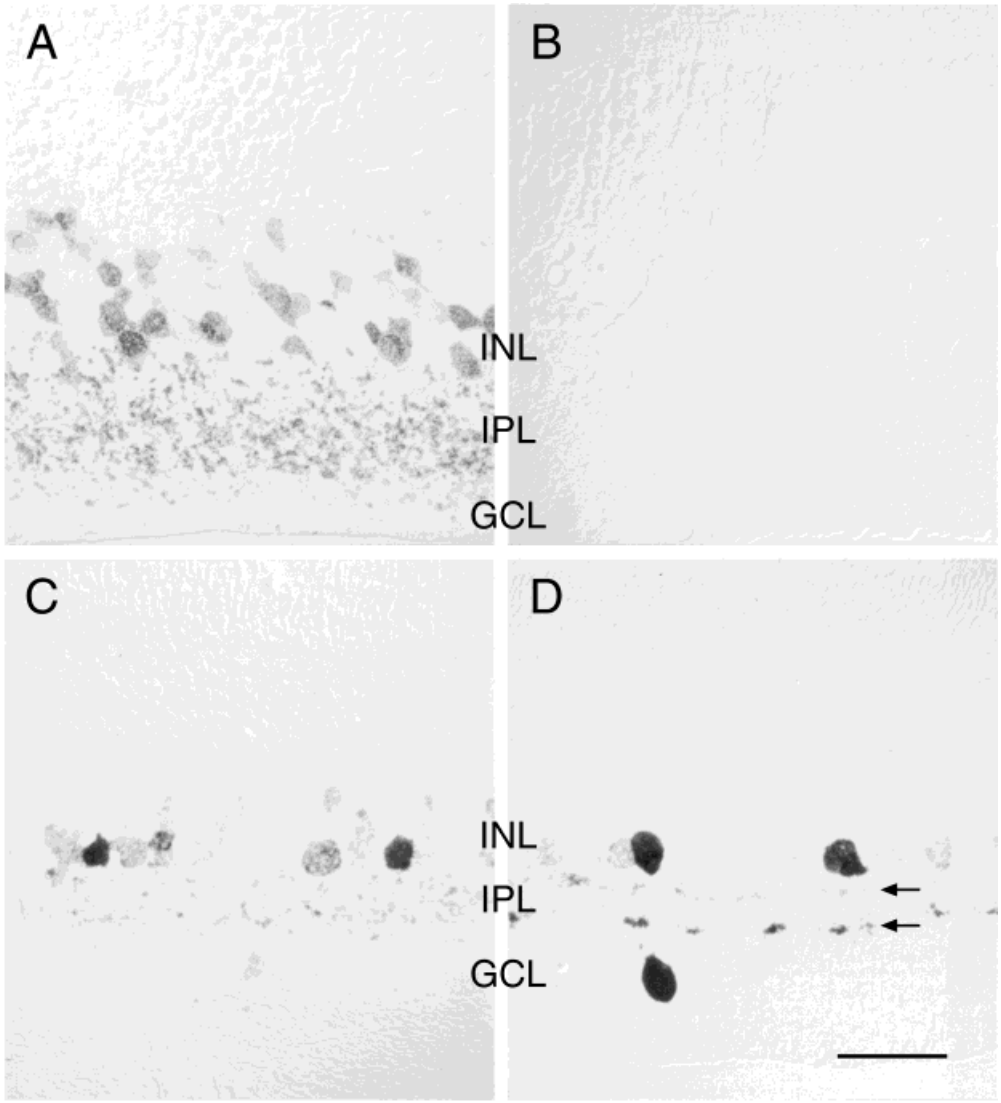

Fig. 1. Effects of sarcosine on glycine immunolabeling of the retina. Transverse resin sections of rat retina (**A**) and rabbit retina (**C**) incubated in control Ames medium show glycine immunoreactivity in subsets of the amacrine cells and bipolar cells. Incubation with excess sarcosine in Ames medium depletes the endogenous glycine in rat retina (**B**) but leads to the intense accumulation of glycine by the cholinergic amacrine cells in rabbit retina (**D**). The two strongly labeled type *a* cholinergic cells in the inner nuclear layer (INL) stratify narrowly in sublamina *a* of the inner plexiform layer (IPL) and the intensely labeled type *b* cholinergic cell in the ganglion cell layer (GCL) stratifies narrowly in sublamina *b* (arrows). Scale bar = 20 μm.

preparations, where the C*a* somata were selectively labeled with high contrast (Fig. 2D), their dendrites still showed much less immunoreactivity than the overlapping C*b* amacrine cells (Fig. 2E,F).

The striking observation of glycine-immunoreactive cholinergic cells in sarcosine-incubated retina was not unprecedented as we had previously observed that the C*b* amacrine somata show elevated levels of glycine immunoreactivity when rabbit retina is incubated in control Ames medium for extended periods (Fig. 2A), although the immunoreactivity is less than that of most glycinergic amacrine cells (Fig. 2B). If the moderate accumulation of glycine in the control medium, which does not contain sarcosine, is mediated by the same mechanism as the intense accumulation of glycine in the sarcosine-containing medium, this would suggest that the glycine accumulation by the cholinergic cells does not depend directly on sarcosine. Rather, the effects of sarcosine appear to arise secondarily from blocking the re-uptake of glycine released from the glycinergic amacrine cells (Pow, 1998). We tested this hypothesis using a variety of strategies, as outlined in the following section.

Mechanism of sarcosine-induced glycine accumulation

The biochemical metabolism of glycine, sarcosine (methylglycine) and choline are closely linked. In principle, surplus choline can be dehydrogenated to form betaine, which can be transmethylated to form dimethylglycine, which in

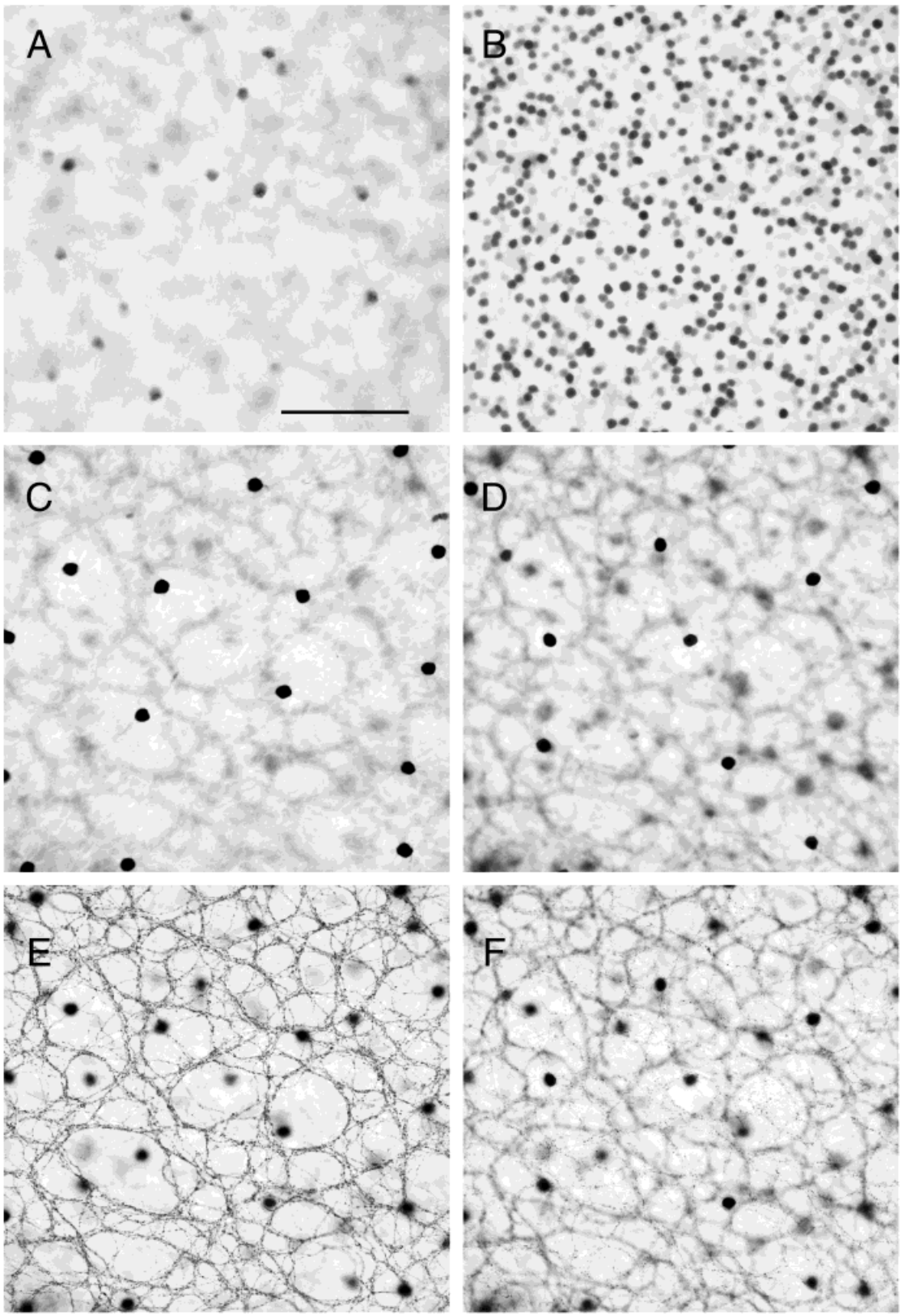

Fig. 2. Glycine immunolabeling of rabbit retinal wholemounts. **A,B:** Tissue incubated in sarcosine-free Ames medium, with the focus on the ganglion cell layer (A) or the inner nuclear layer (B). The elevated levels of endogenous glycine in subsets of the amacrine cells and bipolar cells are retained (B) while the type *b* cholinergic cells show moderate accumulation of glycine (A). **C–F:** Tissue incubated with excess sarcosine in Ames medium shows specific glycine immunolabeling of the type *a* and type *b* cholinergic cells, whose somata are located in the inner nuclear layer (D) and the ganglion cell layer (C), respectively. The cholinergic dendritic plexus in sublamina *b* of the inner plexiform layer is labeled intensely (E) and the cholinergic dendritic plexus in sublamina *a* is labeled moderately (F). Scale bar = 100 μm.

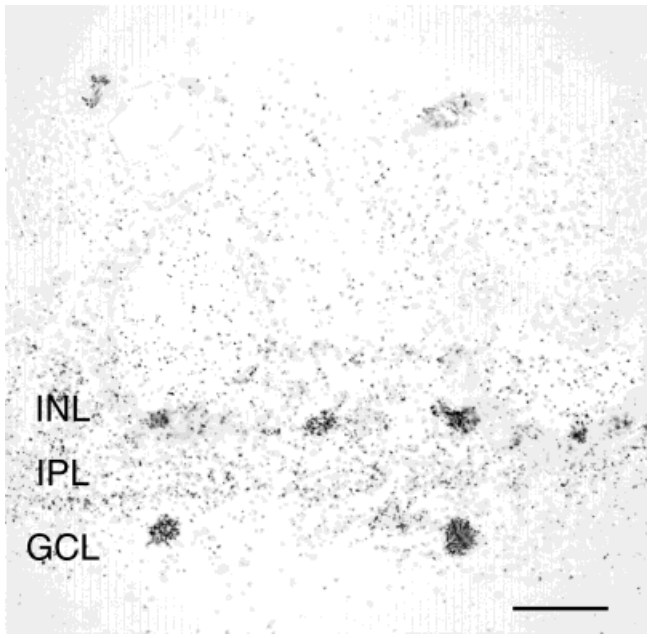

Fig. 3. Sarcosine-induced accumulation of exogenous glycine. Autoradiograph of a transverse section of rabbit retina that was incubated with ^{3}H-glycine and excess sarcosine reveals strong labeling of somata with the size and position of cholinergic amacrine cells. Scale bar = 20 μm.

turn can be successively demethylated to form sarcosine and then glycine:

Choline → Betaine → Dimethylglycine → Sarcosine → Glycine.

This presents a myriad of possibilities of how extracellular sarcosine could raise the intracellular glycine in a cholinergic neuron, with the most obvious candidates requiring uptake of glycine or sarcosine by either a glycine-, sarcosine-, or choline-transporter. We have undertaken experiments to exclude many of these possibilities, but we first describe our positive results that support the simple hypothesis that the elevated levels of glycine in the cholinergic amacrine cells are derived by the uptake of glycine from the extracellular space.

Glycine. The above hypothesis was tested directly by incubating rabbit retina with ^{3}H-glycine and excess sarcosine in Ames medium for 3 hours. Autoradiography of transverse sections of the retina revealed both intense radiolabeling over somata with the size and position of C*b* amacrine cells and moderate radiolabeling over a greater number of somata in the amacrine sublayer of the inner nuclear layer (Fig. 3). Short incubation times of 15–30 minutes were ineffective, although they resulted in strong radiolabeling of amacrine and bipolar somata in the inner nuclear layer when the tissue was incubated without sarcosine (Vaney et al., 1998). This provides further support that the sarcosine-induced accumulation of glycine by the cholinergic amacrine cells is a slow process.

If the raised levels of intracellular glycine are taken-up directly from the extracellular pool, then reducing the extracellular glycine concentration should reduce the glycine uptake by the cholinergic amacrine cells. It was rather surprising, therefore, that these cells still showed strong glycine immunoreactivity when the retina was incubated with sarcosine in glycine-free Ames medium. However, further experiments revealed that incubation of the retina in the glycine-free medium alone for 4–6 hours did not qualitatively affect the normal pattern of glycine immunoreactivity, although the labeling intensity was somewhat reduced. That is, the retina can largely maintain its glycine metabolism when placed in a glycine-free medium, presumably by the efficient re-uptake of glycine released by the amacrine and bipolar cells.

The cholinergic amacrine cells do not express either of the two high-affinity glycine transporters that have been identified in the central nervous system: GLYT1 is expressed by the glycinergic amacrine cells only (Zafra et al., 1995; Vaney et al., 1998; Pow and Hendrickson, 1999) and GLYT2 has not been detected in the retina of the rat or rabbit (Zafra et al., 1995; DV Pow, unpublished observations). This suggests that the cholinergic amacrine cells may accumulate glycine using less selective amino-acid transporters that have a low affinity for glycine. Two such candidates are the alanine-preferring transporter system and the alanine-serine-cysteine transporter system (Shen and Lajtha, 1979). ^{3}H-glycine-uptake experiments on the retina indicated that 90% of the low-affinity uptake can be inhibited by 5 mM alanine, proline or serine (Chin and Lam, 1980; Kong et al., 1980; Marc, 1984). However, none of these amino acids had any significant effect on the sarcosine-induced accumulation of glycine by the cholinergic cells (Fig. 4C), and the retinas showed a normal pattern of glycine immunoreactivity when incubated with 5 mM serine in sarcosine-free Ames medium (not illustrated). Thus the transporter responsible for the sarcosine-induced accumulation of glycine appears to be different from the low-affinity glycine transporters that are commonly used by non-glycinergic neurons to transport glycine and certain neutral amino acids for metabolic purposes. Moreover, the selective labeling of the cholinergic amacrine cells is not consistent with glycine uptake by a transporter that is ubiquitously distributed, leading us to consider transporters that may be selectively expressed by cholinergic neurons.

Choline. The fact that only the cholinergic amacrine cells accumulate glycine in the presence of sarcosine raised the possibilities that surplus choline is converted to glycine, that glycine is taken up by the choline transporter, or that sarcosine is taken up by the choline transporter and then converted to glycine. These possibilities seemed unlikely because the cholinergic amacrine cells in other retinas do not accumulate glycine in the presence of sarcosine, and two simple experiments excluded a role for choline or its transporter. First, incubation of the retina with 1 mM choline in Ames medium for 5 hours did not affect the pattern of glycine immunoreactivity (not illustrated), indicating that the accumulation of surplus choline does not result in glycine formation. Second, incubation of the retina with 1 or 5 mM choline did not prevent the sarcosine-induced accumulation of glycine by the cholinergic amacrine cells (Fig. 4D), indicating that the glycine or sarcosine is not taken-up by the choline transporter, which would be saturated by the excess choline.

Sarcosine. It is clear that sarcosine can be transported in tissues such as the liver and kidney (Glorieux et al., 1971; Ballevre et al., 1991) and GLYT1 would be an

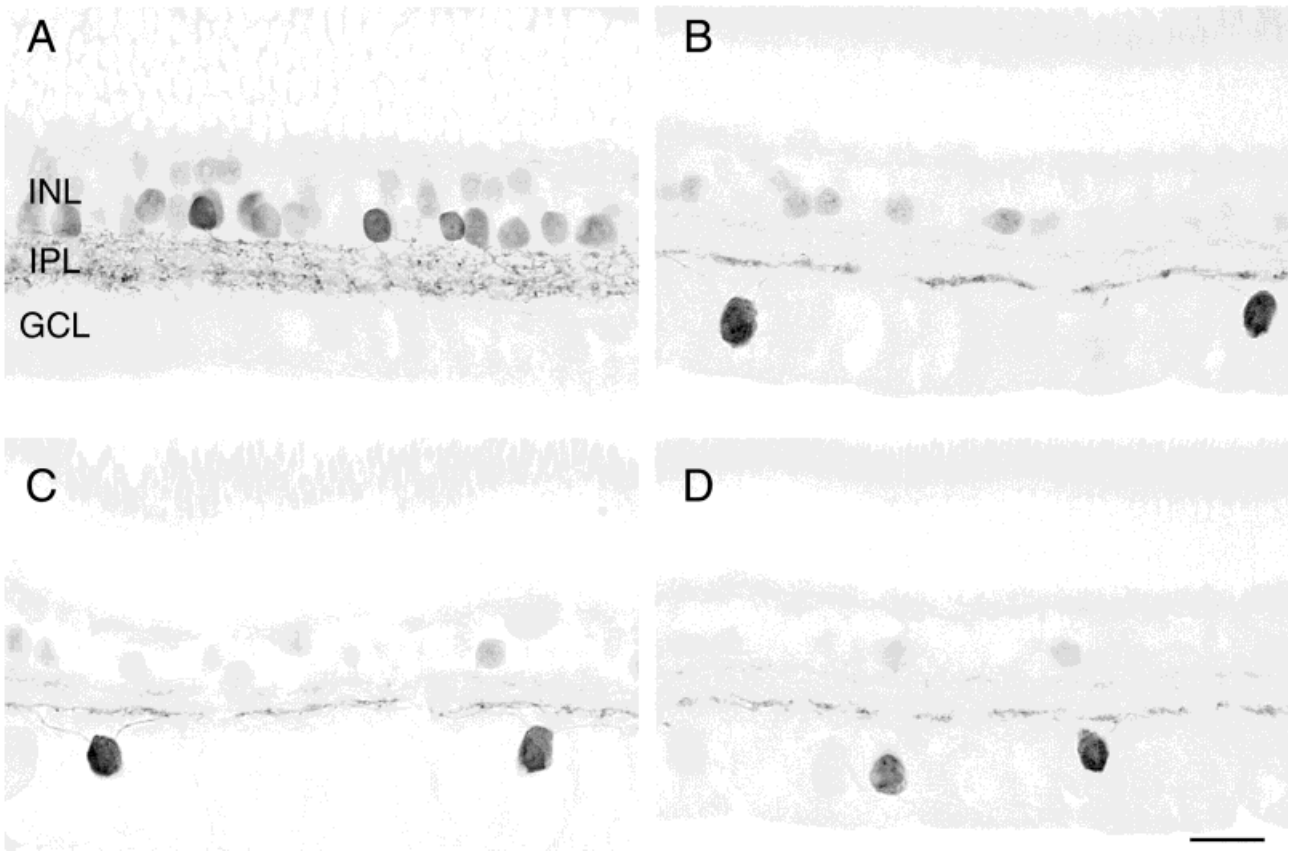

Fig. 4. Negative confocal micrographs of transverse retinal sections immunolabeled for glycine. Pieces of rabbit retina from one eye were incubated for 5 hour under different conditions; they were then fixed, sectioned on a Vibratome, and immunoprocessed identically. **A:** control Ames medium. **B:** 1 mM sarcosine. **C:** 5 mM serine and 1 mM sarcosine. **D:** 1 mM choline and 1 mM sarcosine. Saturation of either the low-affinity glycine transporter with excess serine (C) or the choline transporter with excess choline (D) had no effect on the sarcosine-induced accumulation of glycine by the cholinergic amacrine cells. Scale bar = 20 μm.

obvious candidate to function as a sarcosine transporter in the brain. However, the fact that excess sarcosine depletes the endogenous glycine in the amacrine cells that express GLYT1 indicates that any glycine derived from transported sarcosine is not retained in these glycinergic neurons (Pow, 1998). Indeed, sarcosine dehydrogenase, which catalyzes the conversion of sarcosine to glycine, appears to be absent in the brain (Daley, 1990). If true of the rabbit retina, this would also exclude production of glycine from betaine or dimethylglycine, which are the earlier intermediaries in the postulated choline → glycine metabolic pathway. Betaine is transported by the betaine/GABA transporter BGT-1, but it is not known which retinal neurons express this transporter or if BGT-1 is capable of transporting sarcosine. However incubation of rabbit retina for 5 hours with either 1 mM betaine or 1 mM dimethylglycine did not affect the pattern of glycine immunoreactivity (not illustrated). It is possible that the transporter responsible for the slow accumulation of exogenous ^{3}H-glycine by the cholinergic amacrine cells of the rabbit retina also transports sarcosine but, if the accumulated sarcosine is to contribute to the observed glycine immunoreactivity, it would also be necessary for these cells to express sarcosine dehydrogenase.

Dendritic plexus of cholinergic amacrine cells

Cholinergic amacrine cells. The dendrites of overlapping cholinergic amacrine cells are bundled together in fascicles that form an irregular meshwork surrounding dendrite-free lacunae (Tauchi and Masland, 1985; Brandon, 1987; Famiglietti and Tumosa, 1987). The contribution that a single cholinergic cell makes to the fasciculated dendritic plexus was revealed by injecting Neurobiotin into C*b* amacrine cells in retinal wholemounts that were superfused with 1 mM sarcosine in Ames medium. Following fixation, the Neurobiotin-injected cell was visualized with FITC-tagged streptavidin and the overlapping C*b* dendritic plexus was visualized by glycine immunofluorescence using a Texas Red-tagged secondary antibody. The C*b* amacrine cell illustrated in Figure 5A has a dendritic tree of ≈320 μm diameter and each point on the retina would be overlapped by the dendritic fields of ≈45 neighboring C*b* cells (Tauchi and Masland, 1984; Vaney, 1984); thus the injected cell accounts for only 2–3% of the C*b* dendrites in the field. The lacunae outlined by the fascicles are quite variable in size, ranging from 5–30 μm diameter at this retinal eccentricity.

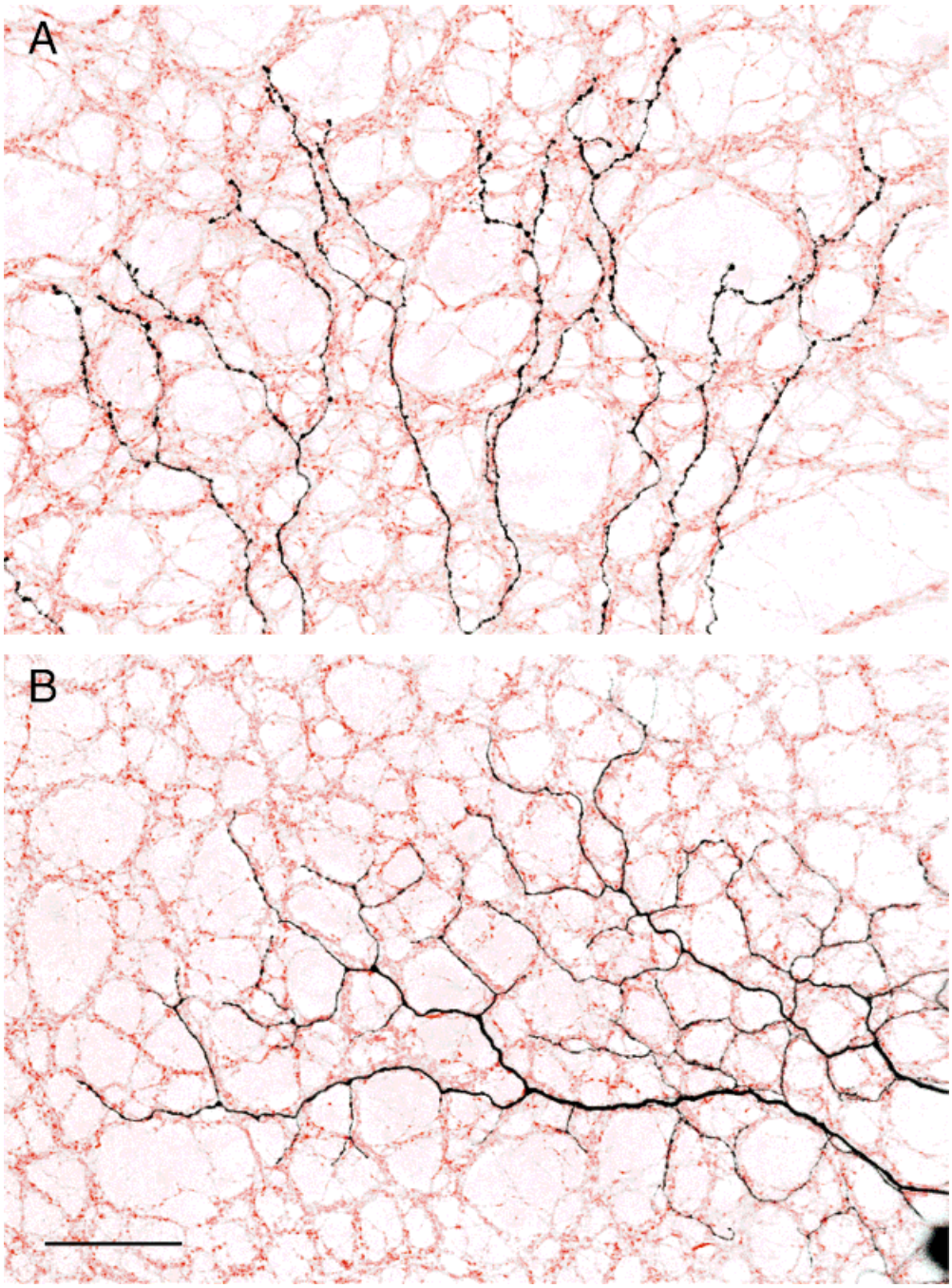

Fig. 5. Confocal micrographs showing the co-fasciculation between Neurobiotin-injected cells (black) and the glycine-immunolabeled cholinergic plexus (red). **A:** part of the dendritic tree of a type *b* cholinergic amacrine cell. **B:** the sublamina *b* arbor of an On-Off direction-selective ganglion cell. Scale bar = 20 μm.

The dendrites of a cholinergic amacrine cell run relatively straight and, thus, the rings of fascicles surrounding the lacunae are formed tangentially by the dendrites of many cells. Some isolated cholinergic dendrites run across the lacunae, and this is apparent for both the Neurobiotin-injected C*b* amacrine cell and the glycine-immunolabeled plexus. Over 95% of the dendrites of injected C*b* cells run in fascicles and this provides a baseline for comparing the dendritic relationships between cholinergic amacrine cells and other types of retinal neurons.

Direction-selective ganglion cells. We previously demonstrated that the cholinergic amacrine cells co-fasciculate with the On-Off direction-selective (DS) ganglion cells by injecting Lucifer yellow into a DS ganglion cell and some of the overlapping C*b* amacrine cells (Vaney et al., 1989), but the dense cholinergic plexus could be only

partially labeled by this method. More recently, we have demonstrated dendritic fasciculation between On-Off DS cells with different preferred directions, by injecting Neurobiotin into two or three overlapping ganglion cells (Vaney, 1994; Amthor and Oyster, 1995). However, these studies did not reveal whether all cholinergic fascicles are associated with dendrites from On-Off DS ganglion cells, and vice versa. The ability to label the whole C*b* dendritic plexus with high contrast using glycine immunocytochemistry provided the means to examine this question directly.

Neurobiotin-injected On-Off DS cells were identified by their distinctive type 1 bistratified morphology; the outer arbor co-stratifies with the C*a* amacrine cells and the inner arbor co-stratifies with the C*b* amacrine cells (Amthor et al., 1984; Famiglietti, 1992). When a single On-Off DS cell was injected with Neurobiotin (Fig. 5B), almost all of the ganglion cell dendrites closely followed the course of a cholinergic fascicle, although many of the fascicles within the dendritic field did not co-fasciculate with the dendrites of the injected ganglion cell. Thus the meshwork of ganglion cell dendrites has a coarser periodicity than the meshwork of cholinergic fascicles, with the looping terminal dendrites usually surrounding several cholinergic lacunae. It seemed probable that the unattached cholinergic fascicles mark the dendritic course of overlapping DS ganglion cells whose preferred directions complement that of the injected ganglion cell.

This was examined directly by injecting Neurobiotin into three overlapping DS ganglion cells with closely spaced somata, which presumably comprised three of the four subtypes of On-Off DS cells present at each retinal location (Oyster and Barlow, 1967; Vaney, 1994). The three ganglion cells are shown at low magnification in Figure 6A, and the sublamina *b* dendrites in the shaded central area are shown at higher magnification in Figure 6B. In the region where all three cells overlap, at the bottom-right of the enlarged field, most of the cholinergic fascicles are associated with one or more dendrites of the injected ganglion cells, but a few cholinergic fascicles in this region remain unattached. It is possible that they mark the dendritic course of the fourth subtype of DS ganglion cell, but this remains to be tested as our attempts to inject four overlapping DS ganglion cells were unsuccessful. In any event, the extent of co-fasciculation between the dendritic plexus of the cholinergic amacrine cells and the meshwork of the On-Off DS ganglion cells is very striking.

Symmetry of dendritic fasciculation. He and Masland (1997) obtained clear evidence that the On-Off DS cells receive symmetrical input from surrounding cholinergic cells by using a laser to selectively ablate C*b* amacrine cells located on either the preferred side of the ganglion cell or the opposite null side (see also Vaney et al., 2000). In contrast, Grzywacz et al. (1997, 1998) have interpreted their own electrophysiological results as indicating that the On-Off DS cells receive strong input from cholinergic cells located on the preferred side and weak input from cholinergic cells located on the null side. In support of this conclusion, the authors cite abstracts of a morphological study showing that "the amount of dendritic material in proximity to DS ganglion cells' dendrites is larger for preferred-side cholinergic cells than for null-side ones" (Amthor and Grzywacz, 1995; Grzywacz et al., 1995). Because this result conflicts with our preliminary findings (Vaney et al., 1989), we went back and re-examined our relevant material, as described below.

Lucifer yellow was injected into an On-Off DS ganglion cell in mid-peripheral rabbit retina and then the Ames medium was drained from the tissue chamber, the retina briefly coverslipped, and the cell's dendritic morphology recorded in a focal series of micrographs (Fig. 7A). Lucifer yellow was then injected sequentially into 10 C*b* amacrine cells whose somata were distributed in a ring around the dendritic tree of the injected ganglion cell (Fig. 7B). Following fixation, the Lucifer yellow in the DS ganglion cell and the cholinergic amacrine cells was converted to an opaque reaction product by photo-oxidation in the presence of diaminobenzidine (Fig. 7C). The dendrites of each injected C*b* amacrine cell extended almost as far as the soma of the injected DS ganglion cell, so that only dendrites on one side of the ganglion cell would make contact with preferred-side cholinergic cells (although the preferred-direction of the ganglion cell is not known). The preparation was examined directly at high magnification (Fig. 7D) and the injected cells were painstakingly reconstructed under a drawing tube (Fig. 7E).

Each point in the dendritic field of the injected ganglion cell was overlapped by the dendritic fields of 3–5 injected C*b* amacrine cells, thus labeling ≈10% of the C*b* dendritic plexus. Although the labeling of the plexus was more complete near the bottom-left of the field, many ganglion cell dendrites on all sides of the soma closely followed the emerging cholinergic fascicles, presenting a pattern that is qualitatively similar to that observed when the whole C*b* dendritic plexus was labeled by glycine immunocytochemistry (Fig. 5B). However, some ganglion cell dendrites at the top-right of the field did not follow cholinergic fascicles, perhaps reflecting the rather sparse labeling of the C*b* dendritic plexus. Consequently, this experiment does not exclude the possibility that there is an asymmetry in the co-fasciculation of preferred- and null-side cholinergic dendrites with a DS ganglion cell.

DISCUSSION

Glycine accumulation by cholinergic amacrine cells

The sarcosine-induced accumulation of glycine by the cholinergic amacrine cells of the rabbit retina was a totally unexpected finding. Sarcosine is a potent competitive inhibitor of the high-affinity glycine transporter GLYT1 (Blasberg and Lajtha, 1966; Guastella et al., 1992) and it depletes the elevated levels of endogenous glycine in the GLYT1-immunoreactive amacrine cells, presumably by preventing the re-uptake of released glycine (Pow, 1998). Sarcosine also depletes the endogenous glycine in the glycine-immunoreactive bipolar cells, which do not express GLYT1 but which appear to obtain their glycine stores by neurotransmitter coupling through gap junctions with glycinergic amacrine cells (Vaney et al., 1998). Like most other GABA-containing amacrine cells, the cholinergic amacrine cells do not express GLYT1 and, in other retinas, sarcosine has no apparent effect on the glycine and GABA levels in the cholinergic amacrine cells (Pow, 1998; Pow and Hendrickson, 1999). The paradoxical effect of sarcosine in the rabbit retina is particularly striking because the induced glycine-immunoreactivity of the cholinergic amacrine cells greatly exceeds the normal glycine-immunoreactivity of the glycinergic amacrine cells.

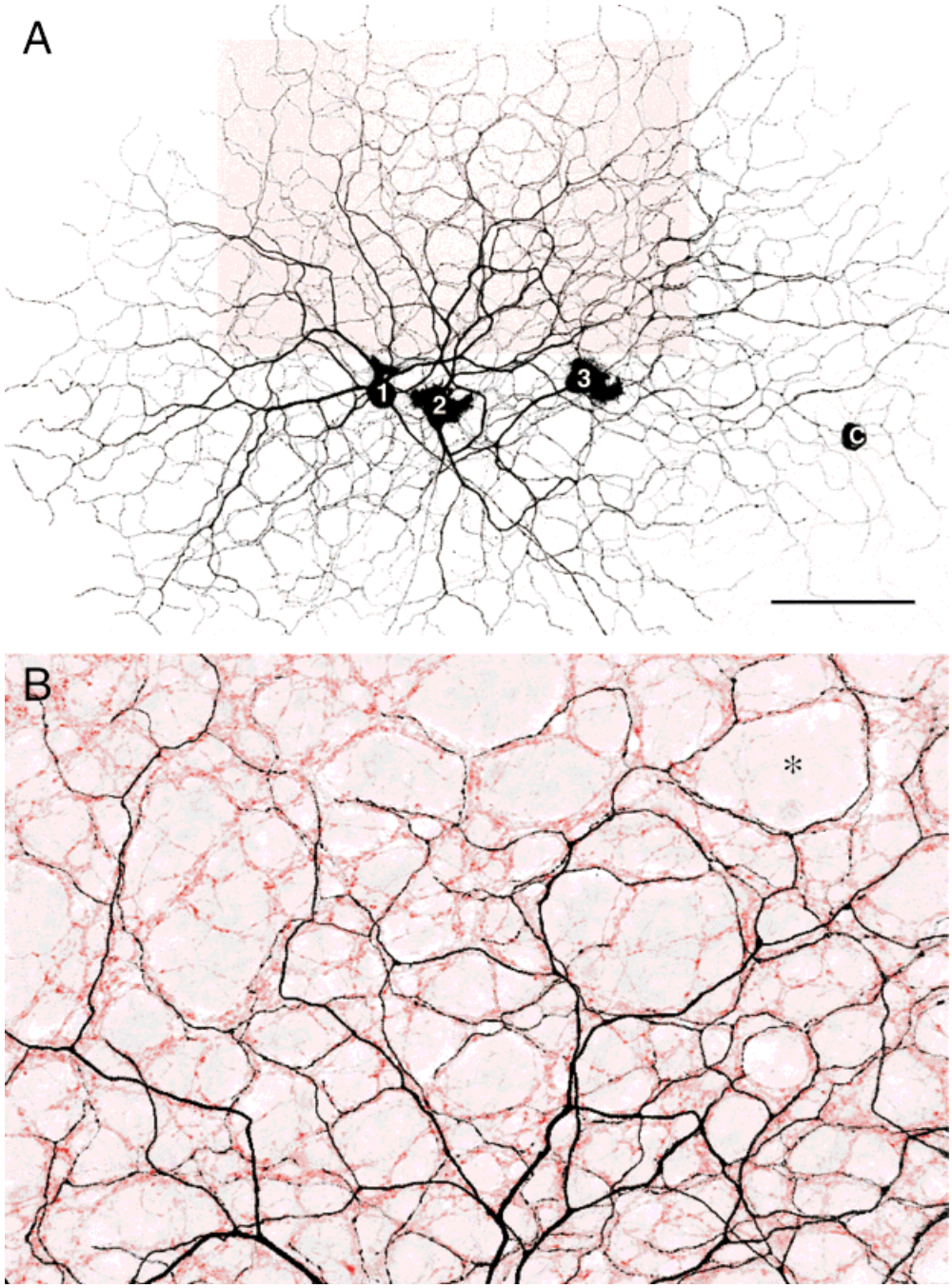

Fig. 6. Co-fasciculation between the dendritic plexus of the cholinergic amacrine cells and the meshwork of the On-Off direction-selective ganglion cells. **A:** confocal Z-series through the full depth of three overlapping ganglion cells that were injected with Neurobiotin (1–3); cell 1 was tracer coupled to a ring of surrounding cells of the same subtype, one of which is included in the field (C). **B:** The sublamina *b* dendrites in the pink area of A are shown at higher magnification, together with the glycine-immunolabeled plexus of the type *b* cholinergic cells; large lacunae (asterisk) are delineated by both the cholinergic fascicles and the ganglion cells dendrites. Scale bar for A = 50 μm.

The available evidence indicates that the sarcosine-induced accumulation of glycine by rabbit cholinergic amacrine cells results from the direct uptake of glycine from the extracellular space. Strong support for this conclusion is provided by the autoradiographic experiments, which showed that exogenous ^{3}H-glycine is slowly taken up by somata with the size and position of cholinergic amacrine cells when the rabbit retina is incubated with sarcosine. Moreover, other incubation experiments provided no support for the alternate hypothesis that sarcosine is converted to glycine within the cholinergic cells.

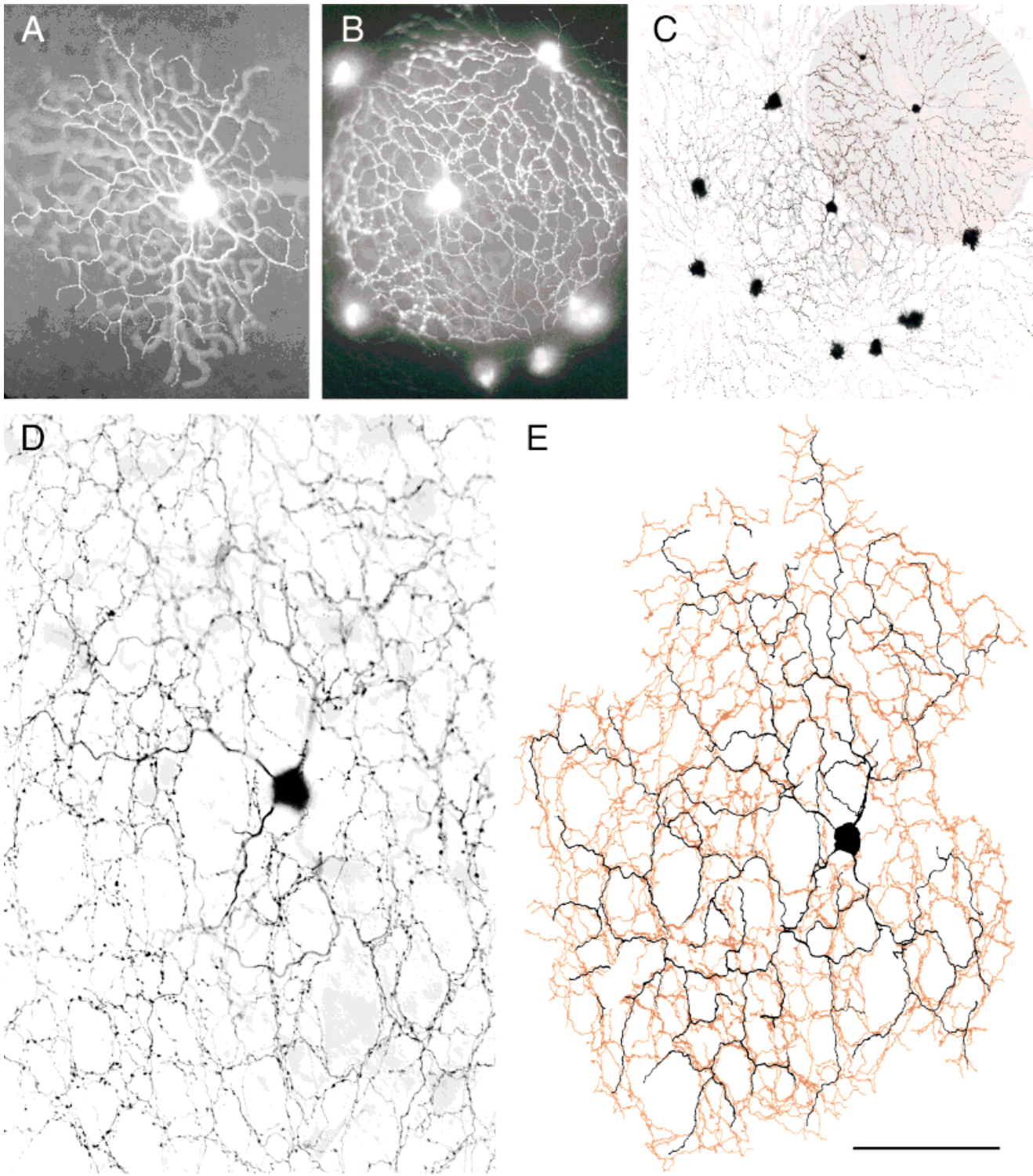

Fig. 7. Symmetry of dendritic fasciculation. Lucifer yellow was injected into an On-Off direction-selective ganglion cell (**A**) and ten surrounding type *b* cholinergic cells (**B**) and then photoconverted to an opaque reaction product (**C**); the dendritic field of a single cholinergic amacrine cell is shaded in pink. The preparation was examined directly at high magnification (**D**) and the injected cells were reconstructed under a drawing tube (**E**). This revealed that many ganglion cell dendrites on all sides of the soma co-fasciculate with the emerging cholinergic fascicles, and thus the ganglion cell dendrites follow cholinergic dendrites that point in any direction. Scale bar for E = 100 μm (A and D after Vaney et al., 1989).

Sarcosine appears to produce its effect by increasing the extracellular concentration of glycine as a result of blocking the re-uptake of glycine by the glycinergic amacrine cells, rather than through any direct effect on a glycine transporter. Support for this conclusion comes from two observations. First, when the rabbit retina is incubated for an extended period in control Ames medium, which does not contain sarcosine, the C*b* amacrine cells show moderate glycine immunoreactivity (Fig. 2A). Second, the C*b* amacrine cells in the rabbit retina begin to show glycine immunoreactivity within 10 minutes of death (Pow and Crook, 1994). Under both conditions, the uptake of released glycine is likely to be impaired due to energetic constraints, thus raising the extracellular concentration of glycine.

The identity of the transporter responsible for the sarcosine-induced uptake of glycine is not known, other than by exclusion. The incubation experiments indicate that the uptake is not mediated by a transporter for choline, sarcosine or the intermediary metabolites, and the high-affinity glycine transporters GLYT1 and GLYT2 have already been excluded. The glycine does not appear to be taken up by ubiquitous low-affinity glycine transporters, which should be blocked by excess alanine, proline or serine (Chin and Lam, 1980). It might appear that rabbit cholinergic amacrine cells express a low-affinity glycine transporter that is not found in other types of retinal neurons or in the cholinergic amacrine cells of other vertebrates. However, it is also possible that the pattern of glycine accumulation reflects a distinctive interplay between the intracellular glycine metabolism of cholinergic amacrine cells and the extracellular glycine metabolism of the avascular rabbit retina. The sarcosine-induced accumulation of glycine appears to be a temperature-insensitive process that proceeds very slowly but concentrates the intracellular glycine to a very high level, particularly in the C*b* amacrine cells.

Dendritic architecture of the cholinergic plexus

The dendritic co-fasciculation that was demonstrated previously by injecting Lucifer yellow into a DS ganglion cell and some of the overlapping cholinergic amacrine cells (Vaney et al., 1989) is shown in this study to be comprehensive. Almost all dendrites of each On-Off DS ganglion cell closely follow the fasciculated dendrites of the cholinergic amacrine cells (Fig. 5B). Moreover, the dendritic meshwork formed by the overlapping subtypes of DS ganglion cells corresponds closely to the meshwork of cholinergic fascicles (Fig. 6B). In fact, the patterns are so similar that it is possible that "the ganglion cell dendrites may be the actual framework upon which the cholinergic lattice is built" (Brandon, 1987). Support for this conclusion comes from an electron microscope study of the rabbit retina, which described fascicles of ganglion cell dendrites as running a "gauntlet" of flanking cholinergic boutons (Famiglietti, 1991). However, our confocal microscope study presents a somewhat different picture in that it is apparent, even when the cholinergic plexus is viewed in only two dimensions, that the dendrites of DS ganglion cells commonly run along the outside of the cholinergic fascicles. This suggests that the cholinergic plexus may develop before the meshwork of ganglion cell dendrites, which is commensurate with the early maturation of the cholinergic system in the retina (Wong and Collin, 1989; Wong, 1995; Feller et al., 1996).

The morphological studies reinforce earlier physiological studies showing that the On-Off DS ganglion cells receive a massive excitatory input from the cholinergic amacrine cells (Masland and Ames, 1976; Ariel and Daw, 1982; Grzywacz et al., 1997; Kittila and Massey, 1997). The cholinergic input appears to be symmetrical and thus could not underlie the asymmetrical generation of direction selectivity in the retina (He and Masland, 1997). Rather, the cholinergic amacrine cells probably facilitate the responses of the ganglion cells to moving stimuli, regardless of the direction of motion.

Three features of the neuronal architecture of cholinergic amacrine cells can be rationalized in this context. First, the proximal-distal segregation of the input and output synapses of cholinergic cells (Famiglietti, 1991) provides the spatial offset that is a prerequisite for motion facilitation. Second, the large dendritic fields of the cholinergic cells ensures that the facilitatory mechanism is responsive to both small and large displacements. The extensive dendritic-field overlap is not redundant because each cholinergic cell that provides input to a local region of a ganglion cell's receptive field would be most responsive to a different vector of motion (Vaney, 1990). Third, the dendritic co-fasciculation of the cholinergic amacrine cells and the four subtypes of On-Off DS ganglion cells would enable each cholinergic dendrite to contact several subtypes of ganglion cells efficiently (Vaney, 1994).

Although the facilitatory cholinergic input to the On-Off DS ganglion cells appears to be symmetrical (He and Masland, 1997), the strong null-direction inhibition ensures that the facilitation is demonstrable only for movements with a component in the preferred direction (Grzywacz and Amthor, 1993). Consequently, the difference between the preferred- and null-direction responses is significantly enhanced by the cholinergic input. Thus the DS ganglion cells respond much more strongly to moving stimuli than to stationary flashed stimuli (Vaney et al., 2000).

Glycine immunolabeling of the cholinergic plexus may prove useful for ultrastructural investigation of the synaptic mechanism of direction selectivity. It would enable the cholinergic amacrine cells to be selectively labeled in glutaraldehyde-fixed tissue, thus optimally preserving the synaptic morphology. Moreover, double immunolabeling against both glycine and GABA would enable the cholinergic dendrites to be distinguished from other GABAergic dendrites, which probably mediate the null-direction inhibition that underlies direction selectivity (He and Masland, 1997; Vaney et al., 2000).

ACKNOWLEDGMENT

We thank Charles Nelson for reconstructing Figure 7E.

LITERATURE CITED

Ames A, Nesbett FB. 1981. In vitro retina as an experimental model of the central nervous system. J Neurochem 37:867–877.

Amthor FR, Grzywacz NM. 1995. Morphological and physiological basis of starburst-ACh amacrine input to directionally selective (DS) ganglion cells in rabbit retina. Soc Neurosci Abstr 20:217.

Amthor FR, Oyster CW. 1995. Spatial organization of retinal information about the direction of image motion. Proc Natl Acad Sci USA 92:4002–4005.

Amthor FR, Oyster CW, Takahashi ES. 1984. Morphology of on-off direction-selective ganglion cells in the rabbit retina. Brain Res 298:187–190.

Ariel M, Daw NW. 1982. Pharmacological analysis of directionally sensitive rabbit retinal ganglion cells. J Physiol (Lond) 324:161–185.

Ballevre O, Buchan V, Rees WD, Fuller MF, Garlick PJ. 1991. Sarcosine kinetics in pigs by infusion of [1-^{14}C]sarcosine: use for refining estimates of glycine and threonine kinetics. Am J Physiol 260:E662–668.

Blasberg R, Lajtha A. 1966. Heterogeneity of the mediated transport systems of amino acid uptake in brain. Brain Res 1:86–104.

Bloomfield SA, Miller RF. 1986. A functional organization of ON and OFF pathways in the rabbit retina. J Neurosci 6:1–13.

Brandon C. 1987. Cholinergic neurons in the rabbit retina: dendritic branching and ultrastructural connectivity. Brain Res 426:119–130.

Brecha N, Johnson D, Peichl L, Wässle H. 1988. Cholinergic amacrine cells of the rabbit retina contain glutamate decarboxylase and gamma-aminobutyrate immunoreactivity. Proc Natl Acad Sci USA 85:6187–6191.

Chin CA, Lam DMK. 1980. The uptake and release of [^{3}H]glycine in the goldfish retina. J Physiol (Lond) 308:185–195.

Crook DK, Pow DV. 1997. Analysis of the distribution of glycine and GABA in amacrine cells of the developing rabbit retina: a comparison with the ontogeny of a functional GABA transport system in retinal neurons. Vis Neurosci 14:751–763.

Daley EC. 1990. The biochemistry of glycinergic neurons. In: Ottersen OP, Storm-Mathisen J, editors. Glycine neurotransmission. New York: John Wiley. p 25–66.

Famiglietti EV. 1991. Synaptic organization of starburst amacrine cells in rabbit retina: analysis of serial thin sections by electron microscopy and graphic reconstruction. J Comp Neurol 309:40–70.

Famiglietti EV. 1992. Dendritic co-stratification of ON and ON-OFF directionally selective ganglion cells with starburst amacrine cells in rabbit retina. J Comp Neurol 324:322–335.

Famiglietti EV, Tumosa N. 1987. Immunocytochemical staining of cholinergic amacrine cells in rabbit retina. Brain Res 413:398–403.

Feller MB, Wellis DP, Stellwagen D, Werblin FS, Shatz CJ. 1996. Requirement for cholinergic synaptic transmission in the propagation of spontaneous retinal waves. Science 272:1182–1187.

Glorieux F, Scriver CR, Delvin E, Mohyuddin F. 1971. Transport and metabolism of sarcosine in hypersarcosinemic and normal phenotypes. J Clin Invest 50:2313–2322.

Grzywacz NM, Amthor FR. 1993. Facilitation in ON-OFF directionally selective ganglion cells of the rabbit retina. J Neurophysiol 69:2188–2199.

Grzywacz NM, Amthor FR, Dacheux RF. 1995. Are cholinergic synapses to directionally selective ganglion cells spatially asymmetric? Invest Ophthalmol Vis Sci 36:865.

Grzywacz NM, Tootle JS, Amthor FR. 1997. Is the input to a GABAergic or cholinergic synapse the sole asymmetry in rabbit's retinal directional selectivity? Vis Neurosci 14:39–54.

Grzywacz NM, Amthor FR, Merwine DK. 1998. Necessity of acetylcholine for retinal directionally selective responses to drifting gratings in rabbit. J Physiol (Lond) 512:575–581.

Guastella J, Brecha N, Weigmann C, Lester HA, Davidson N. 1992. Cloning, expression, and localization of a rat brain high-affinity glycine transporter. Proc Natl Acad Sci USA 89:7189–7193.

Hampson ECGM, Vaney DI, Weiler R. 1992. Dopaminergic modulation of gap junction permeability between amacrine cells in mammalian retina. J Neurosci 12:4911–4922.

Hayden SA, Mills JW, Masland RM. 1980. Acetylcholine synthesis by displaced amacrine cells. Science 210:435–437.

He S, Masland RH. 1997. Retinal direction selectivity after targeted laser ablation of starburst amacrine cells. Nature 389:378–382.

Kittila CA, Massey SC. 1997. Pharmacology of directionally selective ganglion cells in the rabbit retina. J Neurophysiol 77:675–689.

Kong YC, Fung SC, Lam DM. 1980. Postnatal development of glycinergic neurons in the rabbit retina. J Comp Neurol 193:1127–1135.

Maranto AR. 1982. Neuronal mapping: a photooxidation reaction makes Lucifer yellow useful for electron microscopy. Science 217:953–955.

Marc RE. 1984. The role of glycine in retinal circuitry. In: Morgan WW, editor: Retinal transmitters and modulators: models for the brain. Florida: CRC Press. p 119–158.

Masland RH, Ames A. 1976. Responses to acetylcholine of ganglion cells in an isolated mammalian retina. J Neurophysiol 39:1220–1235.

Masland RH, Mills JW. 1979. Autoradiographic identification of acetylcholine in the rabbit retina. J Cell Biol 83:159–178.

Masland RH, Mills JW, Hayden SA. 1984. Acetylcholine-synthesizing amacrine cells: identification and selective staining by using radioautography and fluorescent markers. Proc R Soc Lond B Biol Sci 223:79–100.

Menger N, Pow DV, Wässle H. 1998. Glycinergic amacrine cells of the rat retina. J Comp Neurol 401:34–46.

Oyster CW, Barlow HB. 1967. Direction-selective units in rabbit retina: distribution of preferred directions. Science 155:841–842.

Pow DV. 1998. Transport is the primary determinant of glycine content in retinal neurons. J Neurochem 70:2628–2636.

Pow DV, Crook DK. 1993. Extremely high titre polyclonal antisera against small neurotransmitter molecules: rapid production, characterisation and use in light- and electron-microscopic immunocytochemistry. J Neurosci Methods 48:51–63.

Pow DV, Crook DK. 1994. Rapid post-mortem changes in the cellular localisation of amino acid transmitters in the retina as assessed by immunocytochemistry. Brain Res 653:199–209.

Pow DV, Hendrickson AE. 1999. Distribution of the glycine transporter glyt-1 in mammalian and nonmammalian retinae. Vis Neurosci 16: 231–239.

Pow DV, Vaney DI. 1998. Blockade of glycine transport depletes retinal neurons of glycine and reveals a glycine-accumulating system in rabbit cholinergic amacrine cells. Invest Ophthalmol Vis Sci 39:S686.

Pow DV, Wright LL, Vaney DI. 1995. The immunocytochemical detection of amino-acid neurotransmitters in paraformaldehyde-fixed tissues. J Neurosci Methods 56:115–123.

Sershen H, Lajtha A. 1979. Inhibition pattern by analogs indicates the presence of ten or more transport systems for amino acids in brain cells. J Neurochem 32:719–726.

Tauchi M, Masland RH. 1984. The shape and arrangement of the cholinergic neurons in the rabbit retina. Proc R Soc Lond B Biol Sci 223:101–119.

Tauchi M, Masland RH. 1985. Local order among the dendrites of an amacrine cell population. J Neurosci 5:2494–2501.

Vaney DI. 1984. "Coronate" amacrine cells in the rabbit retina have the 'starburst' dendritic morphology. Proc R Soc Lond B Biol Sci 220:501–508.

Vaney DI. 1990. The mosaic of amacrine cells in the mammalian retina. Prog Retinal Res 9:49–100.

Vaney DI. 1991. Many diverse types of retinal neurons show tracer coupling when injected with biocytin or Neurobiotin. Neurosci Lett 125: 187–190.

Vaney DI. 1994. Territorial organization of direction-selective ganglion cells in rabbit retina. J Neurosci 14:6301–6316.

Vaney DI, Young HM. 1988. GABA-like immunoreactivity in cholinergic amacrine cells of the rabbit retina. Brain Res 438:369–373.

Vaney DI, Peichl L, Boycott BB. 1981. Matching populations of amacrine cells in the inner nuclear and ganglion cell layers of the rabbit retina. J Comp Neurol 199:373–391.

Vaney DI, Collin SP, Young HM. 1989. Dendritic relationships between cholinergic amacrine cells and direction-selective retinal ganglion cells. In: Weiler R, Osborne NN, editors. Neurobiology of the inner retina. Berlin: Springer. p 157–168.

Vaney DI, Gynther IC, Young HM. 1991. Rod-signal interneurons in the rabbit retina. 2. AII amacrine cells. J Comp Neurol 310:154–169.

Vaney DI, Nelson JC, Pow DV. 1998. Neurotransmitter coupling through gap junctions in the retina. J Neurosci 18:10594–10602.

Vaney DI, He S, Taylor WR, Levick WR. 2000. Direction-selective ganglion cells in the retina. In: Zanker J, Zeil J, editors. Processing visual motion in the real world. Berlin: Springer, in press.

Wong RO. 1995. Cholinergic regulation of $[Ca^{2+}]_i$ during cell division and differentiation in the mammalian retina. J Neurosci 15:2696–2706.

Wong RO, Collin SP. 1989. Dendritic maturation of displaced putative cholinergic amacrine cells in the rabbit retina. J Comp Neurol 287: 164–178.

Wright LL. 1998. Amacrine cells of the rabbit retina. PhD thesis, University of Queensland.

Wright LL, Macqueen CL, Elston GN, Young HM, Pow DV, Vaney DI. 1997. The DAPI-3 amacrine cells of the rabbit retina. Vis Neurosci 14:473–492.

Zafra F, Aragôn C, Olivares L, Danbolt NC, Giménez C, Storm-Mathisen J. 1995. Glycine transporters are differentially expressed among CNS cells. J Neurosci 15:3952–3969.