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Morphology of human retinal ganglion cells with intraretinal axon collaterals

BETH B. PETERSON AND DENNIS M. DACEY

Department of Biological Structure, University of Washington, Seattle

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Abstract

Ganglion cells with intraretinal axon collaterals have been described in monkey (Usai et al., 1991), cat (Dacey, 1985), and turtle (Gardiner & Dacey, 1988) retina. Using intracellular injection of horseradish peroxidase and Neurobiotin in *in vitro* whole-mount preparations of human retina, we filled over 1000 ganglion cells, 19 of which had intraretinal axon collaterals and wide-field, spiny dendritic trees stratifying in the inner half of the inner plexiform layer. The axons were smooth and thin ($\sim$2 μm) and gave off thin ($<$1 μm), bouton-studded terminal collaterals that extended vertically to terminate in the outer half of the inner plexiform layer. Terminal collaterals were typically 3–300 μm in length, though sometimes as long as 700 μm, and were present in clusters, or as single branched or unbranched varicose processes with round or somewhat flattened lobular terminal boutons 1–2 μm in diameter. Some cells had a single axon whereas other cells had a primary axon that gave rise to 2–4 axon branches. Axons were located either in the optic fiber layer or just beneath it in the ganglion cell layer, or near the border of the ganglion cell layer and the inner plexiform layer. This study shows that in the human retina, intraretinal axon collaterals are associated with a morphologically distinct ganglion cell type. The synaptic connections and functional role of these cells are not yet known. Since distinct ganglion cell types with intraretinal axon collaterals have also been found in monkey, cat, and turtle, this cell type may be common to all vertebrate retinas.

Keywords: Human retina, Ganglion cell types, Axon collaterals

Introduction

Branching axons in the inner plexiform layer of the retina have been observed in a wide variety of vertebrate species, though the origin of these fibers has not always been clear. Studies in lizard, chicken, dog, monkey, and human (Ramon y Cajal, 1893; Ventura & Mathieu, 1960; Honrubia & Elliott, 1968, 1970) have described branching axons that were thought to be efferent to the retina since they could be traced to the optic disk but did not appear to originate within the retina. Other studies in cat, dog, and calf (Marenghi, 1901), and dog and human (Gallego & Cruz, 1965), observed branching axons originating from cells with somas located in the ganglion cell layer. Gallego and Cruz described the cells they observed as "associational" ganglion cells since they could trace the axon branches to their terminations within the retina, but the cells did not appear to send axons to the optic disk. In cat retina, extracellular injection of horseradish peroxidase (HRP) has revealed the presence of a ganglion cell type with intraretinal axon collaterals whose main axon could be traced to the optic disk (Dacey, 1985). A distinct ganglion cell type with intraretinal axon collaterals has also been found in turtle retina using intracellular injection of HRP (Gardiner & Dacey, 1988). Usai et al. (1991), using the reduced silver technique to stain monkey retinas, found evidence for two different systems of branching axons in the retina. One system is composed of putative centrifugal fibers resembling those described by Honrubia and Elliott in human and monkey retina. The other system comprises a population of axon collateral fibers that originate from ganglion cells whose main axon extends to the optic disk.

In the present study, intracellular injection of HRP and Neurobiotin in an *in vitro* preparation of the intact human retina was used to demonstrate the detailed morphology of ganglion cells with intraretinal axon collaterals. Evidence is presented that these cells represent a distinct morphological type of wide-field, sparsely branching, monostratified ganglion cells with intraretinal axon collaterals that terminate in the inner plexiform layer.

Materials and methods

In vitro isolated retina

The *in vitro* retinal whole-mount preparation and intracellular injection technique have been previously described (Dacey, 1989; Dacey & Petersen, 1992; Dacey, 1993). Human eyes (n = 46, age range 16–82 years) were obtained 90–120 min after death from donors to the Lions Eye Bank at the University of Washington. Eyes were dissected at the corneoscleral junction and the vitreous

Correspondence and reprint requests to: Dennis M. Dacey, Department of Biological Structure, Box 357420, University of Washington, Seattle, WA 98195-7420, USA.

was removed from the eyecup. The retina was dissected free of the sclera and choroid in a continuously oxygenated culture medium (Ames, Sigma Chemical Co., St. Louis, MO) and placed flat, photoreceptor side down, in a superfusion chamber on the stage of a light microscope. Retinas were maintained in the chamber for ~8–10 h with no apparent deterioration in morphology. Following an experiment, retinas were fixed for 2–3 h in a phosphate-buffered fixative (2% glutaraldehyde, 2% paraformaldehyde or 4% paraformaldehyde; 0.1 M, pH 7.4).

Intracellular injection and histology

Ganglion cells were stained *in vitro* with the vital dye, acridine orange, and observed under episcopic illumination. Intracellular injections were made into fluorescing cells with bevelled microcapillary electrodes. Electrodes were filled with Lucifer Yellow (~2%; Aldrich Chemical, Milwaukee, WI) in 20 mM, pH 7.0 MOPS buffer (Sigma Chemical Co., St Louis, MO) and either rhodamine-conjugated horseradish peroxidase (HRP) (~4%; Sigma Chemical Co., St Louis, MO) or Neurobiotin (~4%; Vector Labs, Burlingame, CA). Lucifer Yellow fluorescence in the electrode and acridine orange fluorescence of ganglion cells were observed with the same excitation filter (410–490 nm; barrier filter 515 nm), permitting direct observation of the electrode tip as it penetrated a cell. Lucifer Yellow was passed into an impaled cell to confirm a successful penetration. Ganglion cells were then filled with either rhodamine-conjugated HRP (1–5 nA positive current for 1–3 min) or Neurobiotin (0.1–0.5 nA positive current for 30–60 s).

Following fixation, cells injected with rhodamine-conjugated HRP were revealed using diaminobenzidine (DAB) as the chromogen. Retinas were incubated in the DAB solution (0.1% in 0.1 M phosphate buffer, pH 7.4) for 5 min and then H_2O_2 (0.003%) was added and the retinas further incubated for 3–4 min. Retinas were rinsed in buffer, wholemounted on gelatin-coated slides, air

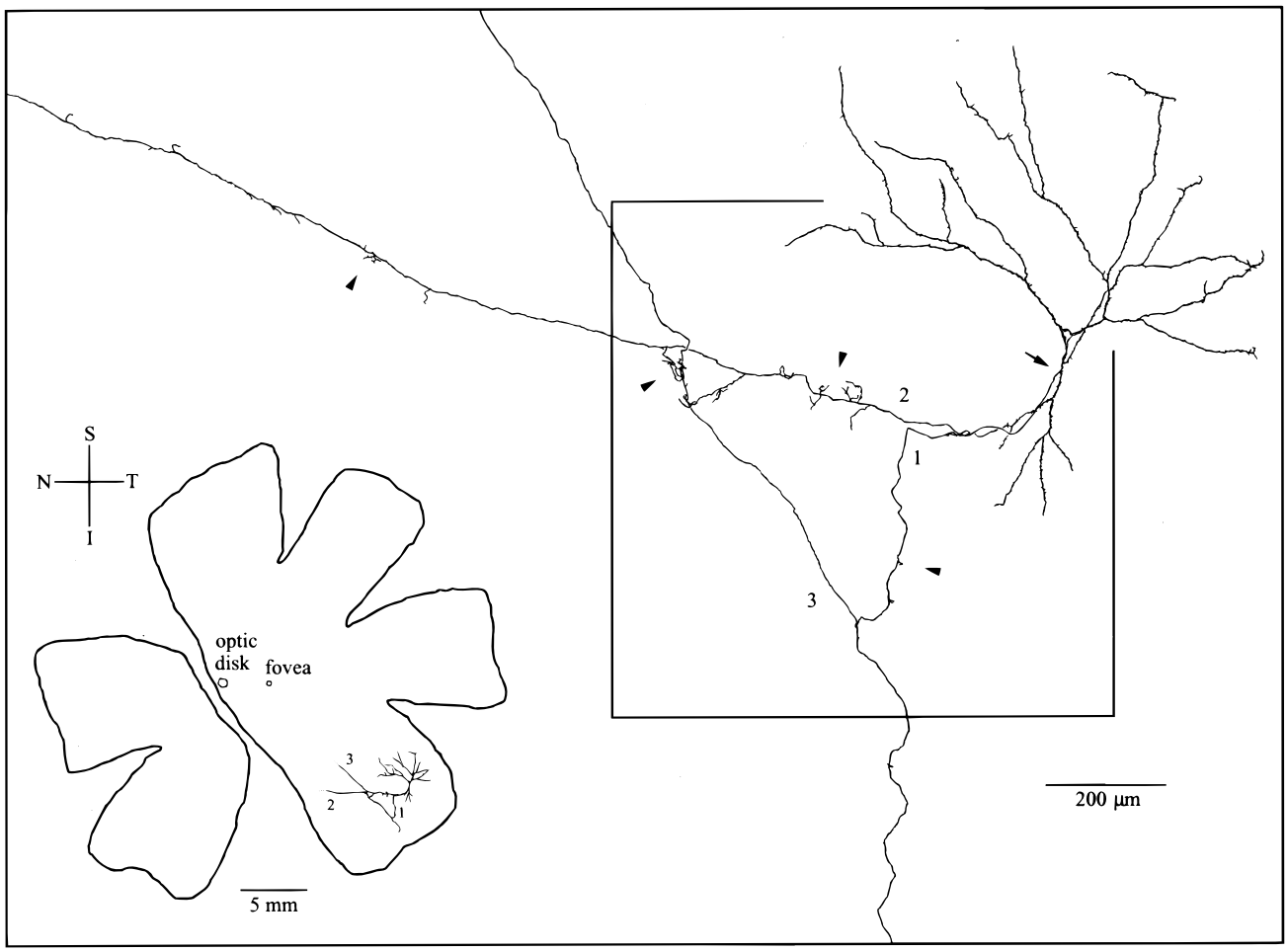

Fig. 1. Camera-lucida tracing of an HRP-filled ganglion cell with axon collaterals. Whole-mount inset at lower left shows the position of the cell in lower temporal retina at an eccentricity of 12.1 mm. The soma is 18 μm in diameter and gives rise to a large, sparsely branching dendritic tree 760 μm in diameter. Arrow indicates the origin of the axon on a primary dendrite. The axon bifurcates twice, forming three branches labelled 1, 2, and 3. Axon branches 1 and 2 lie near the GCL/IPL border. Axon branch 1 extends from temporal to inferior retina then turns in the direction of the optic disk before fading. Axon branch 2 projects in a fairly straight course from temporal to inferior retina. Both axon branches 1 and 2 give off terminal collaterals (arrowheads), present either as short, single processes or in clusters of branched and unbranched processes that extend vertically to terminate in the outer half of the IPL. Axon branch 3 is smooth with no terminal collaterals and extends towards the optic disk in the OFL. All three axon branches are ~2 μm in diameter and can be followed for several millimeters before they fade. The boxed area is shown enlarged in Fig. 2.

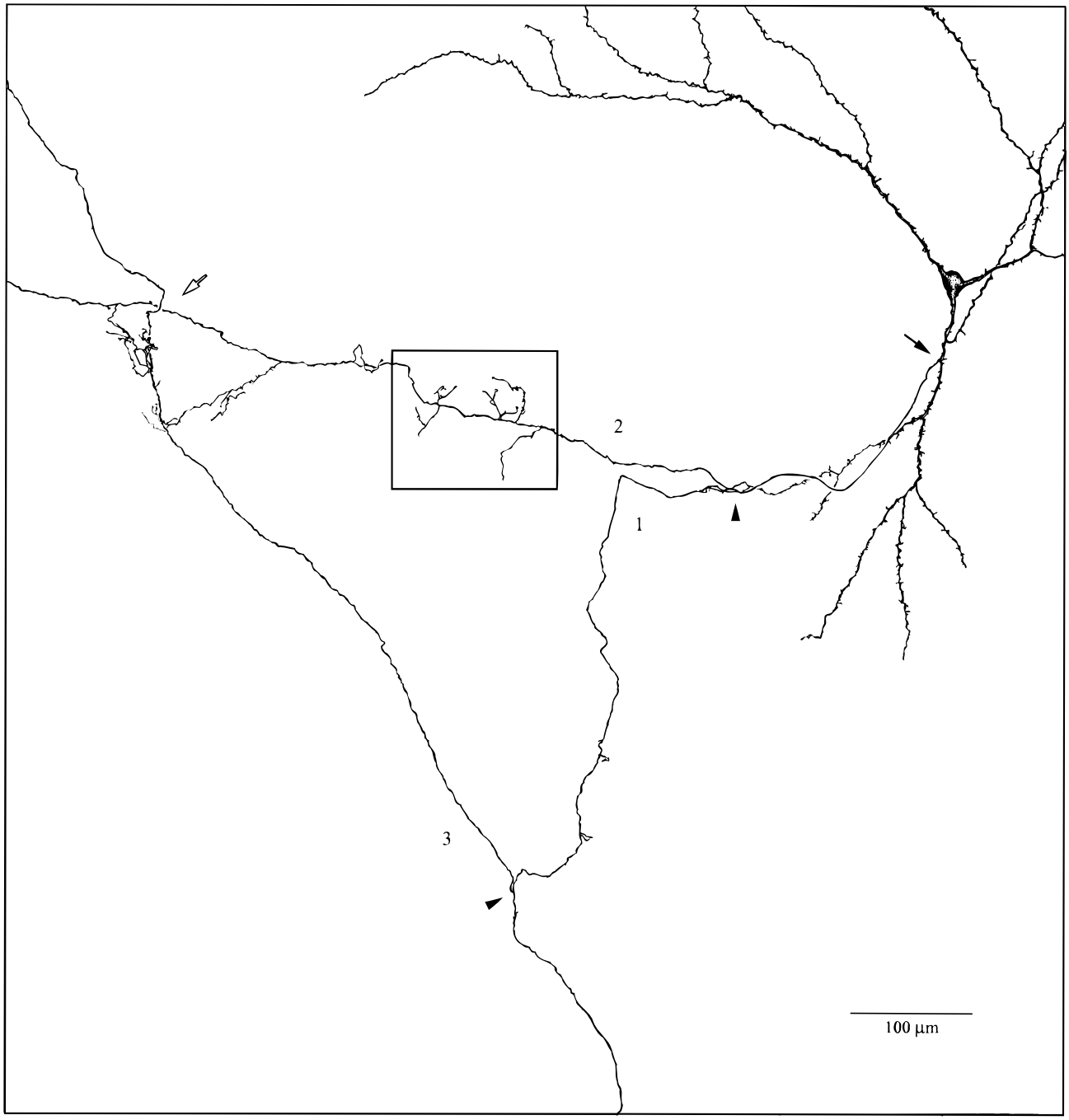

Fig. 2. Enlarged view of boxed area of Fig. 1. The axon branches are labelled 1, 2, and 3, as in Fig. 1. The origin of the axon (arrow) is more easily seen, along with the two branch points (arrowheads). Axon branch 3 courses towards the optic disk in the OFL and crosses axon branch 2 (open arrow) near the site of two terminal collaterals that originate from axon branch 2 and extend vertically to terminate in the outer half of the IPL. Note the spiny appearance of the dendrites, which are more easily seen in this enlarged view. Terminal collaterals in boxed section are shown magnified in Fig. 3.

dried for a few hours, then dehydrated in a graded alcohol series, cleared, and coverslipped.

Cells injected with Neurobiotin were revealed by HRP histochemistry using the Vector ABC protocol (Vector, Elite kit). Retinas fixed in 4% paraformaldehyde were placed in 0.5% triton X-100 (0.1 M phosphate buffer, pH 7.4) for 3 h at room temperature, then incubated in buffer containing the Vector avidin-biotin-HRP complex for 3 h. The retinas were rinsed in buffer for 1 h before performing standard HRP histochemistry using DAB as the chromogen as described above.

Data analysis

The location of every injected ganglion cell relative to the foveal center was recorded for each retina. A camera-lucida tracing of the dendritic tree (total magnification, 480× or 960×) and an outline

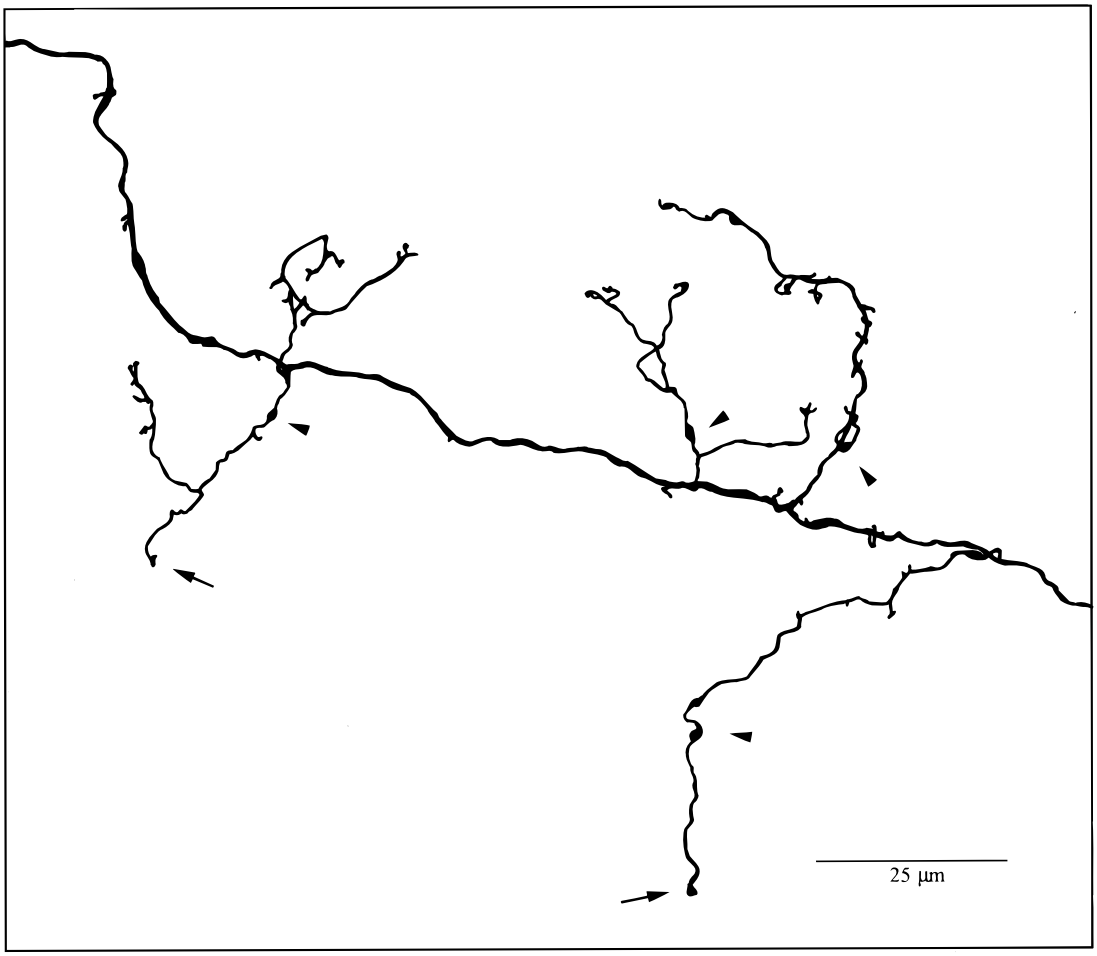

Fig. 3. Magnified view of boxed area of Fig. 2, showing a cluster of terminal collaterals. Collaterals are less than 1 μm in diameter and range in length from about 20–70 μm. They are dotted with varicosities (arrowheads) and terminate in somewhat flattened lobular boutons about 1–2 μm in diameter (arrows).

of the cell body (total magnification, 1940×) was made for each cell. Dendritic-field diameter was determined by tracing a convex polygon around the perimeter of the traced dendritic field, and the area of this polygon was calculated by using a graphics tablet to enter the outline into a computer. Dendritic-field diameter was expressed as the diameter of a circle with the same area as that of the polygon. Similarly, cell body size was expressed in terms of an equivalent diameter.

Results

Identification of ganglion cells with intraretinal axon collaterals

The data base for this study was derived from a larger data base of intracellularly filled human retinal ganglion cells containing a diversity of morphological types (Dacey et al., 1991). The present study includes 19 well-filled ganglion cells from 14 healthy adult retinas with no indication of injury or disease. Dendritic extent and detail could be clearly seen in all the cells and the axons could be followed across the retina for several millimeters. Axon collateral-bearing ganglion cells were unambiguously identified within a larger, diverse group of monostratified ganglion cells on the basis of thin, varicose, terminal processes that branch from the main axon or from secondary axon branches, and on the basis of soma size, dendritic-field size, branching, and stratification.

Axon morphology

The camera-lucida drawing of Fig. 1 illustrates a ganglion cell with intraretinal axon collaterals. In six of the cells examined, the axon had 2 to 4 axon branches. The axon of the cell in Fig. 1 originates on a primary dendrite, indicated by the arrow, and has three branches, labeled 1, 2, and 3. All three axon branches are ~2 μm in diameter and can be followed for several millimeters before fading. Axon branches 1 and 2 lie near the border of the ganglion cell layer (GCL) and the inner plexiform layer (IPL), and give off short collaterals (arrowheads in Fig. 1) that extend vertically to terminate in the outer half of the IPL. Axon branch 1 extends from temporal towards inferior retina, then turns abruptly in the direction of the optic disk before fading. It has fewer terminal collaterals than axon branch 2 and the collaterals are short, unbranched processes with terminal boutons. Axon branch 2 follows a fairly straight course from temporal towards inferior retina, giving off both branched and unbranched terminal collaterals of varying length. Axon branch 3 is smooth with no terminal collaterals and extends

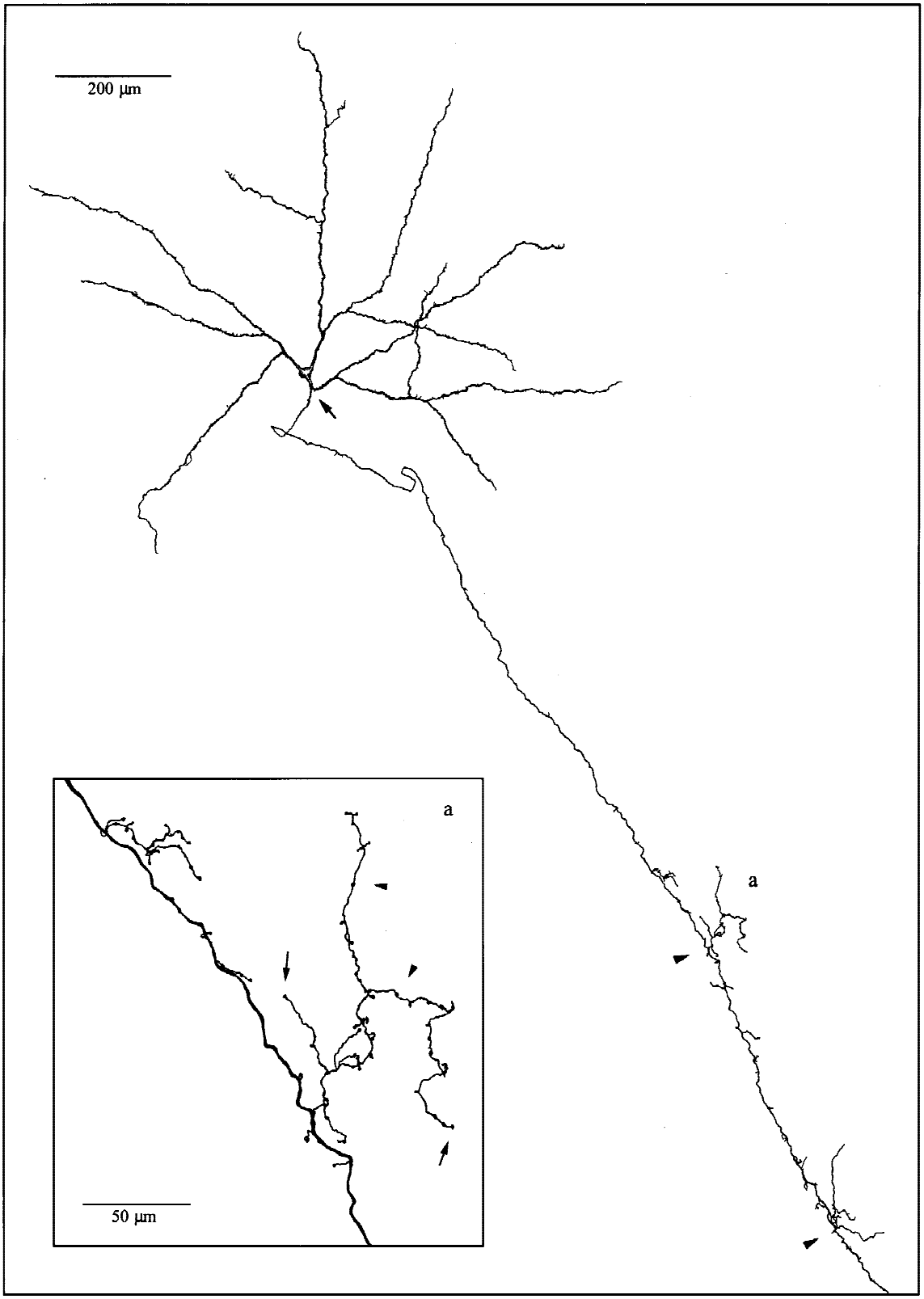

Fig. 4. Camera-lucida tracing of another ganglion cell with axon collaterals. The cell was located in inferior retina at an eccentricity of 11.8 mm. Soma diameter is 20 μm and dendritic-field diameter is 880 μm. Dendrites are 3–5 μm in diameter and are sparsely branched and spiny. Arrow indicates origin of the axon on a primary dendrite. In contrast to the cell shown in Figs. 1 and 2, this cell has only one axon. The axon (2 μm in diameter) lies in the GCL just beneath the OFL and extends towards the optic disk for ~6 mm before fading. Arrowheads indicate several terminal collaterals originating from the axon, with the collaterals marked *a* enlarged in the inset. The collaterals are less than 1 μm in diameter and range in length from 4–150 μm. They are branched and studded with varicosities (arrowheads), and terminate in round or flattened boutons (arrows).

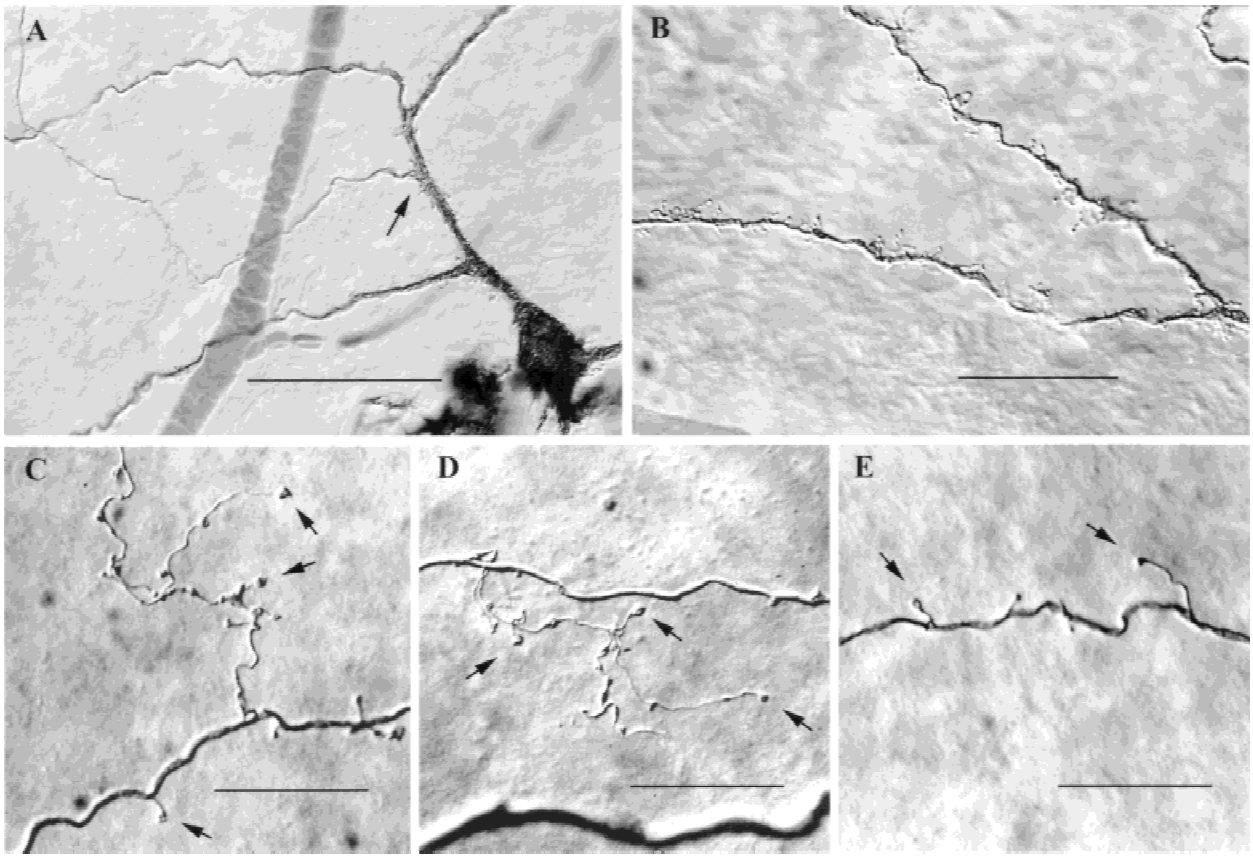

Fig. 5. Photomicrographs of HRP-filled ganglion cells with intraretinal axon collaterals. A: Soma and part of the dendritic tree of a cell located in temporal retina at an eccentricity of 11.6 mm, showing the origin of the axon on a secondary dendrite (arrow). Soma diameter is 21 μm. The dendrite tapers from an initial thickness of about 4 μm. The single, unbranched axon is less than 2 μm in diameter. B: Part of the dendritic tree of another cell. Dendrites taper over their length and are less than 2 μm in diameter in this photomicrograph. They appear crimped and are studded with short, spine-like projections along their entire length. C, D, E: Examples of terminal collaterals from two different cells. The axons are less than 2 μm in diameter, the collaterals less than 1 μm. The collateral in C is branched and varicose, continuing about 600 μm beyond the area shown in the micrograph. The collateral in D is also branched, with a more bushy appearance, and is 55 μm in length, whereas the collaterals in E are unbranched and very short (4–8 μm). The collaterals terminate in round or flattened lobular boutons (arrows) 1–2 μm in diameter. Scale bar for A = 50 μm, for B–E = 25 μm.

towards the optic disk in the optic fiber layer (OFL). The whole-mount inset to Fig. 1 shows the relative position of the cell in lower temporal retina and the direction of axon projection.

Fig. 2 is an enlarged view of the boxed area in Fig. 1, more clearly showing the origin of the axon and its branches, and several short terminal collaterals. As axon branch 3 courses towards the optic disk, it crosses axon branch 2 (open arrow) in the area of two terminal collaterals originating on axon branch 2. Axon branch 2 lies near the GCL/IPL border while its collaterals extend vertically into the outer half of the IPL. Axon branch 3 crosses above these processes in the OFL.

The boxed area of Fig. 2 has been magnified in Fig. 3 to illustrate the terminal collaterals, which form clusters of boutons along the axon branch. The collaterals are studded with varicosities (arrowheads) and have round or somewhat flattened lobular terminal boutons about 1–2 μm in diameter (arrows in Figs. 3, 5C, 5D, and 5E). The processes are thin (<1 μm) and branch repeatedly along their length (Figs. 3, 5C, and 5D), giving a bushy appearance, or are present as unbranched processes of varying length (Figs. 3 and 5D). The terminal collaterals of the cell shown in Figs. 1, 2, and 3 are relatively short ($\sim$3 to $\sim$130 μm). In the group of cells as a whole, terminal collaterals typically were 3 to 300 μm long, though some were up to 700 μm long.

Fig. 4 is a camera-lucida tracing of another ganglion cell with intraretinal axon collaterals. Thirteen cells appeared to have only one primary axon with no secondary axon branches. In Fig. 4, the arrow indicates the origin of a single, thin ($\sim$2 μm), unbranched axon on a primary dendrite. The axon extends from inferior retina towards the optic disk in the GCL just beneath the OFL for 6 mm before fading. Several clusters of terminal collaterals originate from the axon (arrowheads). The inset is an enlarged view of the collaterals marked *a* in the figure. The collaterals are branched and studded with varicosities (arrowheads), and terminate in round or flattened boutons (arrows). About 3 mm from the cell body, the axon gives off a long, thin (<1 μm), somewhat varicose, unbranched collateral (not shown in Fig. 4) that extends for about 2 mm in the IPL before it fades.

In nine of the 13 cells with only a single axon, and in two of the six cells with two or more axon branches, the axons did not appear to extend toward the optic disk. In all of the cells, axons were located either in the OFL or just beneath it in the GCL, or near the GCL/IPL border. Regardless of the location of the axon, the ter-

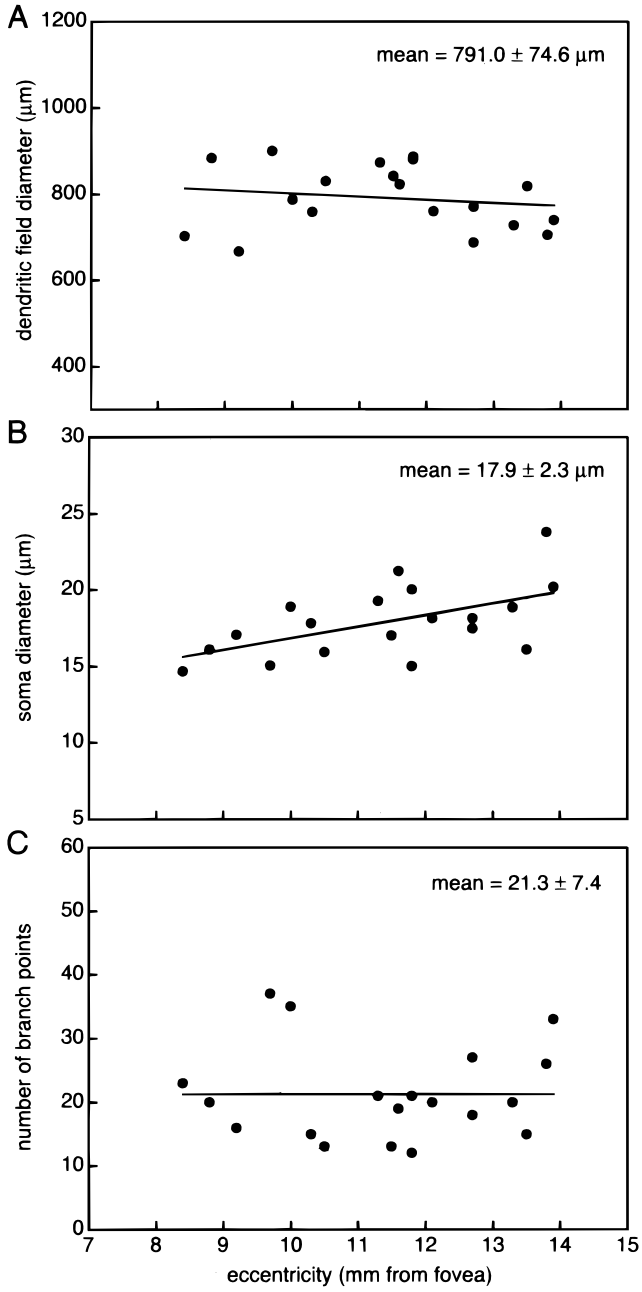

Fig. 6. Dendritic-field diameter, soma diameter, and number of branch points for the 19 axon collateral-bearing ganglion cells, plotted as a function of retinal eccentricity. A: Dendritic-field diameters (range = 667 to 899 μm; mean = 791.0 ± 74.6 μm) show a narrow size distribution with little variation over the eccentricity range sampled. B: Somas are of medium size, from 15–24 μm in diameter, with a mean of 17.9 μm. The scatter plot shows a slight trend towards increasing soma size for the narrow eccentricity range sampled. C: Dendritic trees are sparsely branched. The plot shows a narrow distribution of branch points (range = 12 to 37; mean = 21.3 ± 7.4) with no trend towards increasing or decreasing number of branch points for the eccentricities sampled.

minal collaterals extended vertically to terminate in the outer half of the IPL. Due to the thinness of the peripheral retina, where all 19 cells were located, and to vertical shrinkage as a result of tissue processing, stratification could be determined only in a general way, as being in the outer or inner half of the IPL, without determining the precise lamina of stratification.

Soma and dendrite morphology

All ganglion cells with intraretinal axon collaterals have medium-size somas and large, sparsely branched, spiny dendritic trees monostratified in the inner half of the IPL. Somas are typically elongated and range from 15 to 24 μm in diameter, with a mean of 17.9 μm. The scatter plot of Fig. 6B shows a narrow size distribution over the eccentricities sampled, with a slight trend towards increasing size. The soma gives rise to 2–4 primary dendrites which are initially thick (~3 to 7 μm) but taper gradually over their length. In the scatter plot of Fig. 6A, the range of dendritic-field diameters shows a narrow size distribution (range = 667 to 899 μm; mean = 791.0 ± 74.6 μm) with little variation over the eccentricity range sampled. Since overall ganglion cell density is relatively flat at these peripheral eccentricities, no conclusions can be reached about how the dendritic-field size of this cell type changes at more central eccentricities.

As seen in Figs. 1, 4, and 7A, the dendrites project fairly straight from the cell body. The sparse branching is quantified in the scatter plot of Fig. 6C; the number of branch points ranges from 12 to 37, with a mean of 21.3. The crimped and spiny appearance of the dendrites can be seen in Figs. 1, 4, and 7A, but is best illustrated in the tracing of Fig. 2 and the photomicrograph of Fig. 5B. Spines are typically very short, twig-like projections, often thickening at their termination, and appear along the entire length of the dendrites as single projections or in small clusters.

Discussion

Morphology of ganglion cells with intraretinal axon collaterals

Human ganglion cells with intraretinal axon collaterals resemble cells described in monkey (Usai et al., 1991) and cat (Dacey, 1985). Figs. 7A–7C show examples of axon collateral-bearing ganglion cells from human, monkey, and cat retina, respectively. The human cell in Fig. 7A is typical of cells in the present study. It has a medium-size soma and a spiny, sparsely branched, wide dendritic tree stratified in the inner half of the IPL. The two axon branches give off short terminal collaterals that extend into the outer half of the IPL.

In their study of monkey retina, Usai et al. (1991) described two ganglion cells with wide dendritic fields, elongated, medium-size somas, and long, branched axon collaterals that extended into the IPL, tapering and disappearing at different levels of the IPL. The parent axon of both cells extended towards the optic disk in the OFL and could be traced all the way to the optic disk in one of the cells. The dendrites were smooth, straight, sparsely branched, and stratified in the inner half of the IPL. The camera-lucida tracing of Fig. 7B is from an unpublished study (Dacey) of HRP-filled ganglion cells in monkey (*Macaca nemestrina*) retina and resembles the ganglion cells described by Usai and collaborators. It has an elongated soma and smooth, sparsely branched dendrites stratified in the inner half of the IPL. The primary axon extends towards the optic disk in the OFL and gives rise to a long collateral branch that gives off short, bouton-studded terminal collaterals that extend into the IPL. Usai et al. (1991) did not report seeing bouton-studded terminal collaterals as seen on the human cells (Figs. 3 and 4) and the monkey cell in Fig. 7B. In our preparations, the

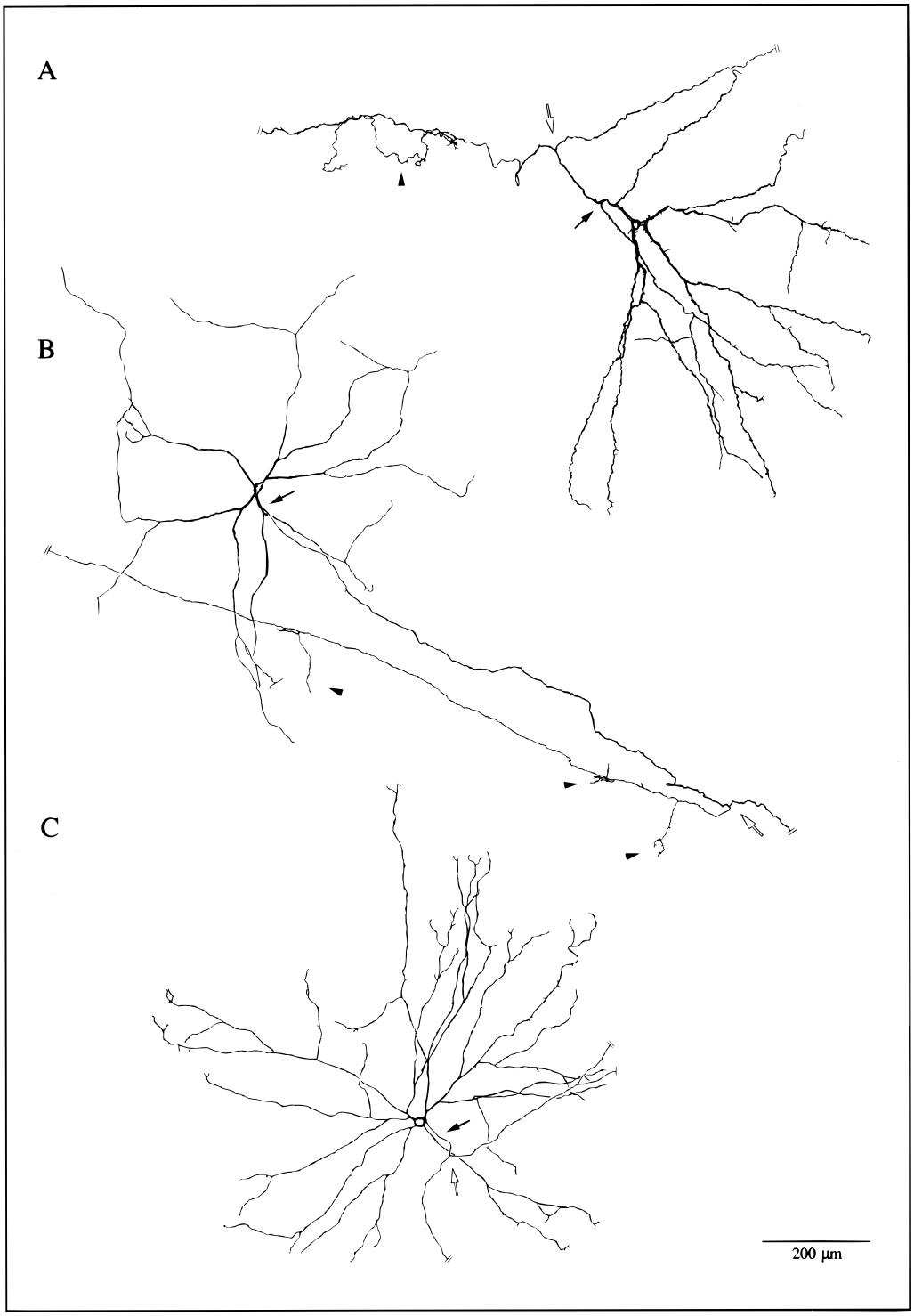

Fig. 7. Camera-lucida tracings of HRP-filled ganglion cells with intraretinal axon collaterals from human (A), monkey (B), cat (C), and turtle (D) retinas. A: Human ganglion cell located in temporal retina 9.2 mm from the fovea. Soma diameter is 17 μm; dendritic-field diameter is 667 μm. Dendrites are crimped and spiny and taper over their length from an initial diameter of ~7 μm. Solid arrow indicates the origin of the axon, which in this cell is initially exceptionally thick (~3 μm in diameter), and tapers to ~2 μm where the axon bifurcates (open arrow). The axon branch to the right in the figure runs near the GCL/IPL border for several millimeters before fading, giving off a few short terminal collaterals. The branch to the left runs for several millimeters towards the optic disk in the GCL near the OFL, giving off numerous clusters of terminal collaterals of varying length, one of which is shown in the tracing (arrowhead). B: Monkey (*Macaca nemestrina*) ganglion cell located in superior retina 10.3 mm from the fovea. Soma diameter is 16 μm; dendritic-field diameter is 787 μm. The dendrites are initially ~4 μm in diameter, sparsely branched, and smooth. The primary axon (solid arrow) is ~1–2 μm in diameter and appears to originate from the soma. It runs in the OFL towards the optic disk, giving off one branch (open arrow) that runs in the opposite direction and gives rise to several terminal collaterals (arrowheads). C: Cat ganglion cell located in temporal retina 5 mm from the *area centralis*. (*caption continued on next page*)

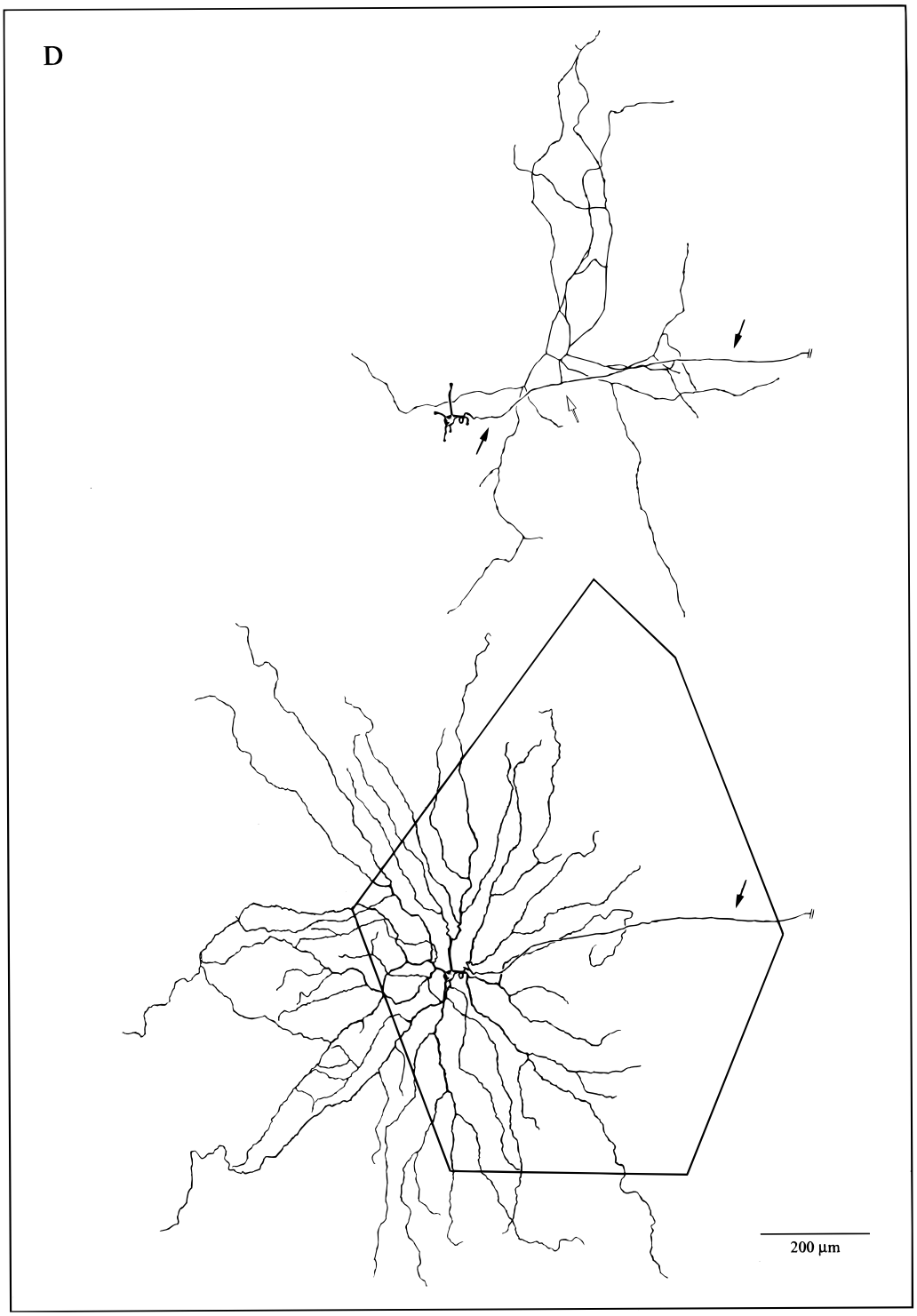

Fig. 7. (*continued*) The soma measures 25 μm along its long axis and 22 μm along its short axis. The dendritic-field diameter is ~1.2 mm. Dendrites are sparsely branched and smooth proximally, becoming varicose distally. Solid arrow indicates the primary axon, which extends towards the optic disk in the lower part of the figure. Open arrow indicates the origin of an axon branch which bifurcates into two branches (upper right in figure) that extend towards dorsal retina and give off clusters of short, terminal collaterals. D: Turtle (*Pseudemys scripta elegans*) ganglion cell located 3.8 mm from the visual streak. Since the axonal arbor partially overlaps the dendritic arbor, the two arbors are shown separately for clarity. The cell with its dendritic arbor is shown in the lower half of the figure. Soma diameter is 20 μm; dendritic-field diameter is ~1.1 mm. The dendrites are ~3 μm in diameter, wavy, moderately branched, and stratify at the outer border of the IPL. The primary axon, indicated by the solid arrow, has a diameter of ~1–2 μm and extends to the optic disk in the OFL. Upper half of the figure shows the soma with truncated dendrites. Solid arrows indicate the primary axon. The open arrow indicates the origin of a collateral branch that descends into the IPL and bifurcates successively, giving rise to an axonal arbor ~744 μm in diameter and monostratified at the inner border of the IPL. The thin axon collaterals are studded with varicosities. The polygon in the lower half of the figure outlines the extent of the axonal arbor and shows its placement relative to the dendritic arbor.

terminal collaterals were extremely thin in comparison to the axon branches. It is possible that such processes were present on the axon branches observed by Usai et al. (1991), but did not stain with the reduced silver technique used by them.

In a study using extracellular injection of HRP in cat retina (Dacey, 1985), 21 intraretinal terminal axons with a characteristic morphology were labeled. In one case, the terminal axons were traced to their origin on a ganglion cell, shown here in Fig. 7C. The cell has a round soma and a large, sparsely branched dendritic tree that is smooth proximally and becomes varicose distally. Similar to the human and monkey cells, the dendrites stratify in the inner half of the IPL. The primary axon gives rise to two branches, one extending to the optic disk in OFL and the other bifurcating into secondary branches that give off short terminal collaterals as they extend dorsally in the IPL. The terminal collaterals were thin, often branched, varicose processes with flattened terminal boutons, very similar to those seen in human retina and illustrated in Figs. 3 and 4.

The cat cell (Fig. 7C), the monkey cell (Fig. 7B), and the monkey cells described by Usai et al. (1991), lack the dendritic spines found in human cells. In other respects, including soma size, axon branching and collateral morphology, dendritic branching, field size, and stratification, the human, monkey, and cat cells are strikingly similar and appear to represent a single distinct morphological cell type.

Ganglion cells with intraretinal axon collaterals have also been observed in turtle retina (Gardiner & Dacey, 1988). Although the morphology of these cells differs in some respects from cat, monkey, and human cells, we have included a sample from turtle retina for comparison (Fig. 7D), and to illustrate that this cell type may be present in a wide range of vertebrate species. The cell is from 33 HRP-filled axon collateral-bearing ganglion cells used in the Gardiner and Dacey (1988) study of turtle (*Pseudemys scripta elegans*) retina. Axonal and dendritic arbors are shown separately for clarity. The lower half of Fig. 7D shows the cell body and large dendritic tree with smooth, wavy, moderately branched dendrites that stratify at the outer border of the IPL. In the upper half of the figure, the primary axon gives off a collateral branch as it extends to the optic disk in the OFL. The collateral descends into the IPL where it branches successively, forming an arbor of thin, varicose processes stratified at the inner border of the IPL.

Since some amacrine cell types are known to have axons (Dacey, 1989, 1990), we considered the possibility that the axon collateral-bearing cells in the present study are not ganglion cells at all but amacrine cells. The axons of eight cells extended towards the optic disk for several millimeters before fading but could not be traced all the way to the optic disk. In 11 cells, none of the axon branches appeared to extend towards the optic disk. Despite the inability to observe axons interring the optic disk, we rejected the possibility that the axon collateral-bearing cells are amacrine cells for the following reasons. Of the known types of amacrine cells with axonal processes, dopaminergic amacrine cells have large, sparsely branching, spiny dendritic trees. However, in cat and monkey retina, the somas of dopaminergic amacrine cells are located in the inner nuclear layer and are rare in the GCL. The axons of dopaminergic amacrine cells are thin, varicose processes that do not give off short, terminal collateral branches. Furthermore, unlike the cells in the present study, both the dendrites and the axon-like processes of the dopaminergic amacrine cell are narrowly stratified near the outer border of the IPL (Dacey, 1988, 1990). Another type of axon collateral-bearing amacrine cell, the somatostatin-positive amacrine cell, has somas located in the GCL. In human retina, somatostatin-positive amacrine cells are found primarily in inferior retina. The dendrites are smooth, and the axons are thin, beaded processes that extend through the IPL and the OFL (Mitrofanis et al., 1989). In comparison, the cells in the present study were located in temporal, nasal, and inferior retina, the dendrites are spiny, and the axon branches are smooth. Most importantly, however, as discussed above, the cells in the present study are morphologically very similar to axon collateral-bearing ganglion cells in cat (Dacey, 1985) and monkey (Usai et al., 1991), where axons have been traced to the optic disk. Given the thinness of the axon branches (<1 μm), it is possible that not all processes in our preparation were stained. Furthermore, the cells in our study were located in peripheral retina, and HRP staining would not normally extend as far as the optic disk. The morphological homogeneity of the cells with intraretinal axon collaterals in human retina and their close similarity to axon collateral-bearing ganglion cells in cat and monkey strongly argue that these cells represent a single morphologically distinct ganglion cell type.

Since none of the thin axon branches showed complete filling, we were unable to determine the full extent of their lateral projection or the total number of terminals present, so comparison with other long-range retinal cell types was not possible. In the few cells filled with Neurobiotin, we saw no homotypic or heterotypic coupling. Also, it would be important to know how these cells tile the retina, but since only one, or at most three cells were filled in each retina, this could not be determined in the present study.

Functional role of intraretinal ganglion cell collaterals

The functional role of intraretinal ganglion cell axon collaterals has yet to be determined. Stratification of the dendrites of these cells in the inner half of the IPL indicates that they are involved in the ON pathway. The bouton-laden terminal collaterals, however, stratify in the outer half of the IPL, suggesting that the collaterals may be propagating action potentials originating in the primary axon, and contacting inhibitory interneurons involved in the OFF pathway.

In their study of monkey retina, Usai et al. (1991) suggested that ganglion cells with intraretinal axon collaterals in the adult retina may represent a developmental aberration, noting that several ganglion cell types in developing cat retina transiently have axon collaterals that are not present in adult retina (Ramoa et al., 1987, 1988). One might expect developmental errors to be rare in the adult retina. Since the 19 cells in the present study come from 14 adult retinas, it seems unlikely that these cells are imperfectly developed ganglion cells. Furthermore, ganglion cells with intraretinal axon collaterals in the adult retina are morphologically homogeneous, which argues against developmental errors since such errors would be unlikely to select for a single cell type.

Several physiological studies have pointed to possible roles for the axon collaterals of ganglion cells. The wide-spread, horizontal distribution of the intraretinal collaterals suggests they may be involved in long-range lateral interactions such as the periphery effect, described by McIlwain (1964) and Levick et al. (1965). In a study of turtle retina, Marchiafava (1976) found that antidromically activated turtle ganglion cells exhibit a late graded synaptic potential that was attributed to amacrine cells shown to be orthodromically activated by stimulation of the optic nerve. Alternatively, the late graded synaptic potential could also be mediated by ganglion cell axon collaterals contacting other ganglion cells.

The functional roles proposed above could also be performed by laterally projecting amacrine cells. Another role, unique to gan-

glion cells, is suggested by evidence pointing to efferent innervation of the retina (Ramon y Cajal, 1893; Hayes & Holden, 1983). Although centrifugal innervation of the primate retina has not been established, some morphological studies suggest the presence of efferent fibers in monkey and human retina. In monkey retina, Usai et al. (1991) described another system of branching axons comprised of putative centrifugal fibers that are larger in diameter than those of the intraretinal axon collateral system and resemble the fibers described by Honrubia and Elliott (1968, 1970) in human and monkey retina. Axon collateral-bearing ganglion cells project to central targets and make synaptic connections within the retina, suggesting that they may play a role in a feedback system involving centrifugal fibers. An understanding of the functional role of these cells awaits the establishment of their central projections and their synaptic connections within the retina.

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