Retinal Ganglion Cells in Goldfish: A Qualitative Classification into Four Morphological Types, and a Quantitative Study of the Development of One of Them

Peter F. Hitchcock and Stephen S. Easter, Jr.

Division of Biological Sciences, The University of Michigan, Ann Arbor, Michigan 48109-1048, and
The Salk Institute for Biological Sciences, P.O. Box 85800, La Jolla, California 92138

In this paper we describe the dendritic morphology of ganglion cells that have been retrogradely stained with HRP taken up by the cut optic nerve. This technique produces an extensive Golgi-like filling of the cells. From their appearance in the retinal whole-mount, they were classified as four types, according to the sizes of the soma and dendritic field, the thickness of the primary dendrites, and the density of the arbors. Each type was subdivided according to the level(s) of stratification of the dendrites within the inner and outer plexiform layer(s) to yield a total of 15 subtypes (four for three types, three for the other).

The retina of the goldfish grows by a balloon-like expansion, and by the addition of new neurons, in annuli, at the margin. Therefore, a similar cell type may be examined at a variety of stages of development in the same retina, as well as in the retinae from fish of various ages. We have used a computer-assisted microscope to do so, quantitatively, for one large and easily identified subtype.

In small fish (ca. 4 cm long), the number of dendritic branch points, the total dendritic length, and the dendritic field sizes of these cells are constant inside a central zone extending to 70–80% of the retinal radius. The magnitudes of all three numeric descriptors decrease closer to the margin. In large fish (ca. 14 cm long), the central zone extends to more than 90% of the retinal radius, and the same pattern holds. The area of the dendritic fields and the total dendritic lengths are both greater in the central zone of the large fish than in the small, but the number of branches is the same in both. This suggests that once a cell has achieved the “mature” number of dendritic branches, further growth is interstitial. A comparison of dendritic morphologies across the retina shows that the pattern of dendritic outgrowth in peripheral retina is initially directed parallel to the margin, and, later, toward the margin. This suggests that dendritic growth is impeded by the dendrites present in more central retina and proceeds preferentially where they are absent.

Cells of the same age are at different distances from the optic disk in the small and large retinae. In some cases, they have quite different dendritic morphologies. This implies that den-

dritic development depends not only on the age and subtype of the cell, but on extrinsic factors as well. Consistent with this interpretation is the demonstration that the coverage of the retina by this cell remains relatively constant with growth.

How dendritic trees develop a particular size and shape, and how this process is controlled are central issues in the study of neuronal development. The retinal ganglion cell has certain features that make it suitable for the study of dendritic development. Its presynaptic inputs are relatively well described, and its dendritic field is often laminar. The retina may be flattened and whole-mounted, thus facilitating the visualization of the intact cells, without the need to cut sections.

The pattern and duration of the growth of the goldfish retina make it an apt site for the study of the dendritic development of ganglion cells. The goldfish retina, like that of other teleosts (Ali, 1964; Kock, 1982a; Kock and Reuter, 1978a; Lyall, 1957; Müller, 1952; Sandy and Blaxter, 1980) grows throughout the life of the animal (Johns and Easter, 1977; Meyer, 1978). This growth is a result of two phenomena; a balloon-like expansion of the existing retina, which results in an increase in retinal area and a decrease in ganglion cell density (Easter et al., 1977; Johns and Easter, 1977; Kock, 1982a; Kock and Reuter, 1978a), and the addition of new neurons, in annuli, from a ring of neuroepithelium at the retinal margin (Johns, 1977; Meyer, 1978; Müller, 1952; Sharma and Ungar, 1980). As a result of this appositional growth, a ganglion cell's proximity to the margin correlates with its youth; the more central the soma, the older, and the more peripheral, the younger. The relative position of any ganglion cell, therefore, changes over time, from an initial position on the margin, to a steadily more central position, as new cells are added peripherally. Each generation of ganglion cells joins a slightly larger retina than the previous generation had done.

The changes with growth are dramatic. In the goldfish, between ca. 1 and 5 years of age, the number of ganglion cells increases by about 50% (Easter et al., 1981) and the retinal area by about 450% (Johns and Easter, 1977). Electrophysiological recordings have shown that the receptive fields, measured in micrometers on the retina, also enlarged with the growth of the whole animal (Macy and Easter, 1981).

All these observations suggest that a systematic study of the dendritic trees of goldfish ganglion cells might reveal new aspects of dendritic development. We have sought answers to the following questions. (1) How does the dendritic morphology vary as a function of the distance from the optic disk, hence age? (2) How does the dendritic morphology of individual cells change as the retina enlarges? (3) Is the dendritic morphology dependent only on the age of the cell, e.g., is a 1-year-old cell in a 1-year-old fish identical to a 1-year-old cell in a 5-year-old fish? (4)

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Correspondence should be addressed to Dr. Hitchcock, Division of Biological Sciences, The University of Michigan, 830 North University, Ann Arbor, MI 48109-1048.

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Table 1. Body length, lens diameter, and retinae of fish studied

Standard length (cm)	Lens diameter (mm)	Retina(e) examined
3.8	1.7	L
3.9	1.6	L & R
4.0	1.8	R
4.4	1.8	L
4.7	1.9	L & R
4.8	1.9	R
5.0	2.0	R
6.3	2.3	L
6.4	2.4	L & R
6.4	2.5	L
6.5	2.2	L & R
8.3	3.1	L
13.6	3.7	L & R
14.5	4.0	R
14.7	4.1	R
15.2	4.4	L

Does the coverage of the retina by ganglion cell dendrites (Wässle et al., 1981b) change as the retina expands, or does it remain constant, as suggested earlier (Kock, 1982b; Macy and Easter, 1981)?

In order to ask these questions, it was first necessary to visualize and classify ganglion cells. They were visualized by retrogradely transported HRP applied to severed axons in the optic nerve. The system of classification is described. Growth-related changes in the dendritic tree of one large and readily recognizable cell type are described and analyzed.

Some of the results have appeared previously in an abstract (Hitchcock and Easter, 1984).

Materials and Methods

Twenty-two retinae from 16 fish, standard lengths 3.9–15.2 cm, lens diameters 1.6–4.4 mm, were used (Table 1). Most of the quantitative parts of this study were carried out on small (ca. 4.0 cm standard length) or large (> 13.0 cm standard length) fish, and we used the terms "small" and "large" retinae to refer to these two classes of fish. Their approximate ages were 1 and 4 years, respectively (Johns and Easter, 1977). Fish were purchased either from a commercial breeder (Grassfork Fisheries, Martinsville, IN), or from a local pet store.

Surgery and histology

HRP was applied in the following way. The fish were anesthetized by immersion in 0.1% tricaine methanesulfonate, the conjunctiva on the dorsal (or ventral) aspect of the globe was cut, the eye was rotated down (or up), the optic nerve was partially sectioned intraorbitally, and a pledget of Gelfoam soaked in 2–5 μ l of 30% HRP (Miles) in 2% dimethylsulfoxide was placed into the orbit adjacent the cut nerve. The eye was then rotated back into the orbit, the incision was sealed with cyanoacrylate, and the animal was allowed to survive for 2–4 d, depending on its size. Following the survival period, fish were dark-adapted for an hour or more, deeply anesthetized, and killed by cutting the heart. The eye was enucleated, the cornea and lens were removed, and the retina was gently separated from the pigmented epithelium in phosphate buffer. To insure that the entire retina was removed, the annular blood vessel that lies peripheral to the retinal margin was always included. The isolated retina was whole-mounted receptor side down on a gelatinized slide, razor cuts were made to aid flattening, and the perforated plastic film from a Telfa pad was placed over the retina and secured with cyanoacrylate. The "sandwiched" retina (Stell, 1967) was fixed in 2% glutaraldehyde in 0.2 M phosphate buffer (pH 7.6) for 30 min. After fixation the plastic film was removed, the vitreous was gently brushed away, and the retina was allowed to dry briefly (10–20 min) to make it adhere to the slide. It was reacted for HRP using the cobaltous

chloride intensified 3,3'-diaminobenzidine protocol of Adams (1977), dehydrated in a graded series of alcohols, cleared in xylenes, and coverslipped.

Well-filled, relatively isolated cells were drawn with the aid of a camera lucida attachment, with a 100 $\times$ planapo oil objective, at a final magnification of 1250 $\times$. The visual isolation of labeled cells was promoted by cutting the nerve incompletely, so that the labeled cells were restricted to an annulus (Rusoff and Easter, 1980), bordered by unlabeled cells.

One limitation of viewing HRP-filled cells in the retinal whole-mount is that in central retina the HRP-filled optic fiber layer creates a dense optical barrier that obscures the HRP-filled somata below. To visualize cells in the central region of the retina, the optic fiber layer was removed, as follows. After clearing, the retina was rehydrated, and the optic fibers were grabbed at the optic disk with fine forceps and carefully peeled up and away. This produced some damage near the disk, but away from this region, the fiber layer and the overlying inner limiting membrane and superficial blood vessels separated cleanly from the ganglion cell layer. (The goldfish retina is avascular.) This procedure left a short stump of axon on most cells and did not appear to damage the somata or dendrites.

Quantitative analyses

The dendrites of a single subtype of ganglion cell (see below) were digitized using a Zeiss Universal microscope (2000 $\times$ magnification), interfaced with a PDP 11/34 computer through stepping motors on the mechanical controls of the stage and fine focus. Data points were entered sequentially, every 5–10 μ m, along the length of the dendrite, beginning at the soma. The computer provided summaries of the number of branch points and total dendritic length for an individual cell, and generated a drawing (e.g., Fig. 7). Areas of the dendritic fields were measured on these drawings by connecting the distal ends of the dendrites and measuring the enclosed area with a Zeiss, Mop-3 image analyzer.

The cross-sectional areas of the somata were measured at 1450 $\times$ magnification, and the areas of the dendritic fields at either 145 $\times$ or 1450 $\times$, using the camera lucida attachment and the image analyzer.

The ideal way to study the changes in dendritic morphology concurrent with the expansion of the retina would be to stain a ganglion cell in the small retina of a young fish and then reexamine the same cell in the larger retina of the now older fish. Because this is not feasible, we have taken advantage of the constant geometry of the goldfish eye (Easter et al., 1977) and the uniform planimetric density of ganglion cells (Johns and Easter, 1977) to predict the location that a cell in a small retina would occupy in a large retina. Cells of the same subtype at these two locations were then compared. The first step was to choose a ganglion cell in a small retina and establish how many ganglion cells lay within the partial hemisphere central to this cell. [The number of ganglion cells in an entire retina, as a function of lens diameter, was obtained from an earlier study (Easter et al., 1981).] Then, in a larger retina, we found a partial hemisphere that contained the same number of cells as the partial hemisphere in the smaller retina.

The cells of the same class that lie on the edges of the two partial hemispheres are considered to be the same cell at earlier and later stages. This procedure is illustrated in Figure 1. (It is assumed that ganglion cells do not die during growth of the retina.) The cell at position *a* forms an angle, θ (expressed as $R_a/R_{\max} \times 90^\circ$) that defines a partial hemisphere (shaded region of hemisphere and idealized whole-mount) with an area

$$A_p = 2\pi r^2(1 - \cos[R_a/R_{\max} \times 90^\circ]) \quad (1)$$

With constant planimetric density (Johns and Easter, 1977), the ratio of the number of cells in the partial hemisphere (N_p) to the area of that partial hemisphere (A_p) is equal to the ratio of the total number of cells in the retina (N_{tot}) to the area of the whole hemisphere (A_{tot}), as given by

$$N_p/A_p = N_{\text{tot}}/A_{\text{tot}} \quad (2)$$

Solving for N_p , the number of cells in a partial hemisphere, gives

$$N_p = N_{\text{tot}}(1 - \cos[R_a/R_{\max} \times 90^\circ]) \quad (3)$$

By measuring the radial distance from the optic disk to a cell and to the margin on the retinal whole-mount (R_a and $R_{\max}$, respectively; Fig. 1), N_p can be determined for a cell in a small retina for any given value of R_a by using equation (3). Knowing N_p for a cell, one can then predict the distance from the optic disk at which this cell would be found in a

Figure 1. Schematic diagram of a goldfish retina. Shown on the left as a hemisphere, with the margin at the equator and the optic disk at the pole, and on the right as an idealized flattened whole-mount. OD, optic disk. See Materials and Methods for details.

larger retina, by solving for R_a in equation (3). Since the distance from the optic disk is expressed as a ratio: R_a/R_{max} , where R_{max} is the maximal radius along the line through the disk and the cell, it is not crucial that the disk be at the center of the retina.

Results

Ganglion cell morphology

Ganglion cells were classified according to the soma size, the diameter of the primary dendrites, the size of the dendritic field, and the density of processes within the dendritic field, as seen in whole-mounts. The following descriptions are derived from camera lucida drawings of 174 labeled cells in retinæ of all sizes and from observations, without drawings, of hundreds more. Several of the ganglion cell types described below were also seen in retinæ that were stained by methylene blue and the Golgi method. The cells have been divided into four types (1–4) which are further subdivided according to the pattern of stratification of their dendrites within one or both of the plexiform layers. [The classification scheme outlined here is slightly different than that presented in a preliminary report (Hitchcock and Easter, 1984).] The boundaries of the inner plexiform layer (IPL) were taken as the outer edge of the ganglion cell layer and the inner edge of the inner nuclear layer, both of which could be discerned in the whole-mounts, even though unstained. The stained dendrites were assigned to one or more of three levels—inner, middle, and outer. Cell types were subdivided according to the level in the IPL in which their dendrites stratified. All types and subtypes were present in retinæ of all sizes. Examples of each type are shown in Figures 2 and 3, the former from a large retina, the latter from a small one. In the next few paragraphs, each cell type and subtype is described. All the numbers are derived from cells in central retina, and none have been corrected for shrinkage. (Linear shrinkage was determined to be less than 20%.)

The Type 1 cell (Fig. 2, cells 1.2 and 1.3, and Fig. 3A) has a large soma (small fish: $108 \pm 36 \mu\text{m}^2$, $n = 20$; large fish: $278 \pm 47 \mu\text{m}^2$, $n = 20$; means $\pm$ SD and number of samples) from which radiate two to five thick, smooth, straight primary dendrites that form a large dendritic field (small fish: $0.16 \pm 0.03 \text{ mm}^2$, $n = 15$; large fish: $0.56 \pm 0.08 \text{ mm}^2$, $n = 17$) with a moderate density of processes. There are four subtypes, with dendrites

(1.1) unistratified in the inner IPL, (1.2) unistratified in the outer IPL, (1.3) within each of the three strata of the IPL, and (1.4) within the middle and outer IPL.

The Type 2 cell (Fig. 2, cells 2.1 and 2.3, and Fig. 3B) has a small soma (small fish: $38 \pm 7 \mu\text{m}^2$, $n = 20$; large fish: $57 \pm 12 \mu\text{m}^2$, $n = 20$) and one to three thin primary dendrites that form a small dendritic field (small fish: $0.011 \pm 0.003 \text{ mm}^2$, $n = 8$; large fish: $0.026 \pm 0.004 \text{ mm}^2$, $n = 8$) with a high density of processes. The dendrites of these cells often possess *en passant* and terminal swellings, as well as swellings at branch points. Dendrites often give rise to retroflexive branches. Type 2 cells also fall into four subtypes, with dendrites (2.1) unistratified in the inner IPL, (2.2) unistratified in the outer IPL, (2.3) bistratified in the inner and outer IPL, and (2.4) unistratified in the middle IPL.

The Type 3 cell (Fig. 2, cells 3.2 and 3.2, and Fig. 3C) has a small soma (small fish: $49 \pm 11 \mu\text{m}^2$, $n = 20$; large fish: $89 \pm 27 \mu\text{m}^2$, $n = 20$), and one to three thin, smooth, straight primary dendrites that taper very little, and form a dendritic field of intermediate size (small fish: $0.10 \pm 0.04 \text{ mm}^2$, $n = 5$; large fish: $0.24 \pm 0.12 \text{ mm}^2$, $n = 8$) with a low density of processes. There are four subtypes, with dendrites, (3.1) unistratified in the inner IPL, (3.2) unistratified in the outer IPL, (3.3) bistratified in the inner and outer IPL, and (3.4) bistratified in the outer IPL and the outer plexiform layer (OPL).

The Type 4 cell (Fig. 2, cells 4.1 and 4.2; Fig. 3D) has a small to medium soma (small fish: $70 \pm 19 \mu\text{m}^2$, $n = 20$; large fish: $109 \pm 30 \mu\text{m}^2$, $n = 20$) and two to five thin dendrites that form a dendritic field of intermediate size (small fish: $0.05 \pm 0.01 \text{ mm}^2$, $n = 4$; large fish: $0.17 \pm 0.05 \text{ mm}^2$, $n = 8$) with moderately dense processes. The overall appearance of Type 4 cells is similar to that of Type 1 cells, but smaller. Type 4 cells have three subtypes, with dendrites (4.1) unistratified in the inner IPL, (4.2) within the middle and outer IPL, and (4.3) within the inner and outer IPL.

The dendrites of the bistratified Type 2 and 3 cells form discrete layers of processes that are restricted to the inner and outer levels of the IPL, with clear break points in between (Fig. 4, A–D). In contrast, the multistratified Type 1 and 4 cells contribute dendrites to two or more levels of the IPL, with some branches restricted to a single level and others more diffusely stratified.

Figure 2. Camera lucida drawings of representative examples of the four types, and some subtypes, of ganglion cells seen in the goldfish retina. The two numbers give type and subtype; e.g., 1.2 is Type 1, Subtype 2. See text for classifications of subtypes. All cells were from the central region of the retina from a large fish (14.5 cm standard length). Cells are oriented as if the optic disk is at the bottom of the figure and the margin is at the top. Axons are marked with arrowheads.

Of the four cell types, Types 1–3 are the most distinct, and can be readily identified in a population of HRP-stained cells. Isolated Type 4 cells are more difficult to identify, owing to their similarity to Type 1 cells. When adjacent, however, Type 1 and 4 cells can be distinguished by the larger soma, thicker primary dendrites, and much larger dendritic field of the Type 1 cell.

The cell types described above are distinguished by differences in their morphology, however, they also share some common characteristics. All may have dendritic appendages, i.e., short spines (less than 15 μm long) and fine filamentous or hair-like processes, often with *en passant* and terminal swellings (filled arrowhead, Fig. 3A). All may have short spines or filopodia-like processes originating from the soma (Fig. 4A). The dendritic membrane at branch points on the proximal dendrites is frequently webbed, as shown by the arrow in Figure 3A (see also Fig. 3C). Many cells have short spines on the proximal regions of the axon. The axons of many cells originate from a proximal dendrite, sometimes several cell diameters removed from the soma. Somata are generally located eccentrically in the dendritic field, usually toward the optic disk (Fig. 2). Finally, examples

of all four cell types were found with their somata displaced into the amacrine cell layer.

We cannot estimate the relative numbers of these four cell types, because in those retinal regions where most or all of the cells were labeled, it was impossible to connect a soma and its dendrites unambiguously. The only one of the four types recognizable from its soma alone is Type 1. Its relative abundance was estimated in two retinæ, one small and one large, by counting labeled ganglion cells in four areas, roughly midway between disk and margin, one in each quadrant. The values were very similar in all cases; the overall abundance was 1.3% (see also Kock and Reuter, 1978a).

Numbers of displaced ganglion cells were estimated similarly. They were relatively more abundant in the large retina (4.2%) than in the small (0.9%). These differences may or may not be significant, and we have not attempted to evaluate that possibility. The important point is that displaced ganglion cells are a small minority of the ganglion cell population.

To exclude the possibility that the HRP-staining may have systematically missed one or more types of ganglion cells, sev-

Figure 2. (Continued.)

eral HRP-labeled retinæ were counterstained for Nissl substance with toluidine blue. HRP-filled and Nissl-stained cellular profiles were drawn at several locations at $1450\times$ magnification and counted. The fraction of cells not stained by HRP varied unsystematically within individual retinæ. Figure 5 shows two representative fields from small and large retinæ in which the HRP filling was judged to be most complete. In both, the cells stained by Nissl alone (dark profiles) were systematically smaller than the HRP-filled cells (clear profiles), had sparse and uniformly stained cytoplasm, and were irregularly shaped. Cells of this description are usually identified as glia (Kock, 1982a; Kock and Reuter, 1978a; Stone, 1978; Wässle et al., 1975). The number of cells with abundant cytoplasm, a pale nucleus, and a nucleolus, i.e., presumptive neurons, that were not stained with HRP, were less than 1% of the HRP-labeled cells. They were probably displaced amacrine cells. We infer that, when the conditions for labeling by HRP were optimal, all ganglion cells were

labeled. The occurrence of unlabeled ganglion cells elsewhere is probably a result of uncontrolled factors, probably having to do with local concentrations of HRP in the nerve. We conclude that it is unlikely that this technique systematically excluded some types of ganglion cells. It is possible that we missed some types because none of their representatives were sufficiently well isolated to be drawn and identified.

Dendritic development of the subtype 1.2 cell

We have used a computer-assisted microscope to study quantitatively the dendritic morphology of subtype 1.2 cells, an example of which is shown in Figures 2 (cell 1.2) and 3A. This cell subtype was selected because it is very distinct, is readily stained, and can be unambiguously identified in retinæ of different sizes and at different distances from the optic disk in the same retina. It has two or three very thick primary dendrites that arise, by a gradual transition, from a large soma that often

Figure 3. Photomicrographs of each of the four types of ganglion cells. The dark background in these photomicrographs is a result of the overlying optic nerve fibers and blood vessels. The optic disk is up and to the right. *A*, Partial dendritic field of a Type 1.2 cell. *Arrow*, Webbing seen at many dendritic branch points of this and other cell types (e.g., see *C*). *Filled arrowhead*, A filamentous appendage that is common to all ganglion cell types. *Open arrowhead*, The out-of-focus soma. *B*, Type 2.1 cell. This cell type has thin primary dendrites, and a high density of processes within a small dendritic field. *C*, Partial dendritic field of a biplexiform Type 3 cell (3.4). The dendrites of this cell stratify within the outer IPL and the OPL. *Arrow*, A fine dendritic branch that leaves the IPL (and plane of focus) to stratify within the OPL with a much more restricted dendritic field. *Open arrowhead*, The out-of-focus soma. *D*, Example of a Type 4.2 cell. *Small arrowheads*, Some of the dendrites of this cell. Dendritic fields of this type are intermediate in size between Types 1 and 2 (*A* and *B*, respectively). All cells are from the central region of a retina from a small fish (4.4 cm standard length). Scale bar, 50 μ m for *A*, *C*, and *D*; 25 μ m for *B*.

has the bulk of its cytoplasm displaced into the IPL. The dendritic field of these cells is very large, and is stratified, in a planar fashion, in the outermost IPL. Three descriptors were chosen to quantify its dendritic arbor: the number of branch points, the total dendritic length (not including "dendritic appendages"), and the area of the dendritic field. The four questions posed in the introduction are treated now, in the original order.

How does dendritic morphology vary with the distance from the optic disk?

The nasal left retina from a small fish (standard length, 3.9 cm; lens diameter, 1.6 mm, ca. 1 year old) was divided into concentric zones, each equivalent to 10% of the distance from the optic disk to the margin (Fig. 6). The central two zones were not examined because of damage caused during the dissection. In each of the peripheral eight zones, the dendrites of three subtype 1.2 ganglion cells (circles in Fig. 6) were digitized (see Materials and Methods), and examples of computer-generated drawings of one cell from each zone are given in Figure 7. They show that across the central retina, the dendritic fields are roughly circular and similar in both size and complexity, but nearer

the margin, they become less complex, smaller, and decidedly noncircular, with the major axis oriented parallel to the margin. This qualitative asymmetry near the margin was initially described in the crucian carp, a congener of the goldfish, by Kock and Reuter (1978b). The quantitative data are shown in the three graphs of Figure 8. The average number of branch points, the total dendritic length, and the sizes of the fields are constant (within a considerable range of variation) for cells within the area bounded by 70–80% of the retinal radius (the central 49–64% of the retinal area), and all three numeric descriptors decrease toward the margin.

The goldfish retina can therefore be divided into two concentric zones: a central one where the dendritic trees of these cells are circular (Fig. 7), and quantitatively similar (Fig. 8); and a marginal one in which trees nearer the margin have progressively more elongated shapes, fewer dendrites, smaller fields, and smaller total dendritic lengths. These observations suggest that young ganglion cells initially send out dendrites in the direction parallel to the margin and, over time, lengthen and branch predominantly perpendicularly to the margin until they have reached maturity at about 20–30% of the distance from the

Figure 4. High-power photomicrographs of a bistratified Type 2 cell in the central retina of a small fish. This series of photomicrographs illustrates the discrete layering of this cell's dendrites within the inner and outer layers of the IPL. *A*, Soma in ganglion cell layer (arrow). Filled arrowhead, An erythrocyte within a blood vessel. Open arrowhead, Appendages commonly seen originating from the somata of HRP-filled ganglion cells. *B*, Dendrites stratified in the inner IPL. *C*, Dendrite-free layer between the inner and outer layers of the IPL. Arrow, A dendritic branch ascending toward the outer layer of the IPL. *D*, Dendrites stratified in the outer layer of the IPL. Note that the soma is increasingly out of focus in *A–D*. In each photomicrograph, the optic disk is toward the lower left. Scale bar, 30 μ m.

margin to the disk. In this retina, that distance corresponds to about 6 months of retinogenesis. We infer that the cells progressed from birth to maturity in this time. (This estimate of time is based on the assumptions that the daily rate of ganglion cell proliferation was constant over the first year, and that this fish was exactly 1 year old.)

How does the dendritic morphology of individual cells change as the retina enlarges?

Two retinæ were compared, one from a small fish (4.0 cm long, 1.8 mm lens diameter, 6.4 mm retinal diameter, estimated age: 1 year, estimated number of ganglion cells: 130,000) and one from a large fish (14.5 cm long, 4.0 mm lens diameter, 15.1 mm retinal diameter, estimated age: 4 years, estimated number of ganglion cells: 180,000). In the small retina, subtype 1.2 cells at various distances from the optic disk were located and digitized. Using the procedure outlined in Materials and Methods, the positions that these cells would occupy in the large retina were determined. Figure 9 shows the camera lucida drawings of the two whole-mounted retinæ, with small circles to indicate the locations of the neurons studied. Within one retina, neurons of the same letter lie at the same percentage of the distance from

the optic disk to the margin (e.g., C = 80% in the large, and 98% in the small). Cells designated by the same letter in the small and large retinæ are considered to be the same cell at early and later stages, respectively.

Figure 9 also illustrates the manner of retinal growth as described in the introduction. The area of the small retina, enclosed by the cells at positions C, would, over time, expand to occupy the area enclosed by cells at positions C in the large retina. The annular portion of the large retina between positions C and the margin contains the cells added as the retina grew from small to large.

Examples of cells from each of these locations are shown in Figure 10, and they illustrate the same centropertipheral progression that was noted in Figure 7. Comparisons of the cells at each position in the upper and lower rows of Figure 10 reveal two differences. First, cells in the small retina are more peripheral than their counterparts in the large. Second, the dendritic fields of ganglion cells in the large retina are much larger. This enlargement involves at least two processes: the addition of new branches and the interbranch elongation of existing dendrites. Clearly, both operate near the margin, but, as will be shown below, central retinal growth may involve only interbranch elongation. A third feature, not shown in the drawings, is in the

Figure 5. Camera lucida drawings from regions in a small (left) and large (right) retina in which the HRP filling was judged to be most complete. Filled profiles, Nissl-stained glia-like cells; open profiles, HRP-filled ganglion cells; cross-hatched profile, a neuron that was not filled with HRP. Asterisks, HRP-filled cells that were displaced into the inner nuclear layer. Scale bar, 30 μ m.

proximal dendrites, which are much thicker in the large retina.

The quantitative data for the cells in the two retinæ are illustrated in Figure 11. (Individual values, rather than means, are given, to illustrate their variability.) All four variables are larger for cells in the central retina. Three of them reach a constant value relatively closer to the margin in the large retina than in the small retina. The number of branch points, the dendritic length, and the field size are constant throughout central retina, out to the limit indicated by points B, 92% of the large retinal radius. Thus, the transition zone, which extended 20–30% of the way in from the margin in the small retina, is only about 8%, and perhaps less, in the large retina. Although the relative thickness of this zone is much smaller in the large retina, its absolute width is not so different from that of the small retina. The retinal radii were 3.4 and 7.5 mm, respectively; 20–30% of the former is 0.7–1.0 mm, 8% of the latter is 0.6

mm. Indeed, if the areas of the peripheral annuli are compared, then that of the larger retina is twice as large. If the two transitional zones are compared with respect to the amount of time that each represents, the outer 8% in the large retina corresponds to about 16 months (Fig. 10) versus 6 months estimated for the outer 20–30% of the small retina. These comparisons suggest that the rates of dendritic growth are roughly similar, or at least not very different, in the two retinæ, despite their very different sizes and ages.

The number of dendritic branch points is about the same for cells in the central regions of the two retinæ (Fig. 11A, positions F and G; Mann-Whitney U test: $p > 0.05$). This invariance suggests that once cells have acquired the “mature” number of branches, no more appear. This is consistent with the data from the small retina of Figure 8A, which shows that the value at 25% is about the same as that at 75%. The other three numeric descriptors, however, are larger in the large retina (Fig. 11, B–D). The growth of the soma (Fig. 11D) warrants no further comment, but the increase in the total dendritic length and the dendritic field size can be analyzed to allow some inferences about the process of dendritic development. The average length of dendritic segments between branch points (total dendritic length/number of dendritic segments) for cells included in Figure 11 is greater for the cells in the central plateau of the large retina than in the small (111.1 vs 50.1 μ m). This could be explained by either, or both, of two mechanisms. The simpler is an elongation of the preexisting dendritic segments (interstitial growth). The alternative is the loss of old dendrites and outgrowth of new ones. The average distance between branch points of cells nearer to the margin is also greater in the large retina (compare C and D, lower panel, with A, upper panel, in Fig. 10). Because of the relative youth of this region, we assume that the tissue cannot have stretched appreciably. This implies that whereas dendritic growth is qualitatively similar for cells near the margin of a small and large retina, this growth is quantitatively different.

Dendritic growth can also be described by considering the dendritic tree in relation to the outside visual world that is imaged on the retina. The magnification factors for the two retinæ of Figure 9 are 37 and 82 μ m/deg (20.5 $\times$ lens diameter; Easter et al., 1977) or 1369 and 6724 μ m²/deg². The average areas of dendritic fields in the central regions of the two retinæ are 160,000 and 560,000 μ m², which correspond to 160,000/1369 = 117 deg² in the small and 560,000/6724 = 83 deg² in the large. Therefore, the enlargement of the dendritic field has not matched the retinal growth, so a larger tree (in μ m²) subtends a smaller visual angle (in deg²). Recall that the retina enlarges by two means: stretch of the preexisting tissue and the formation

Figure 6. Camera lucida drawing of part of the whole-mounted left retina from a small fish (3.9 cm standard length). The retina was divided into concentric zones, each equivalent to 10% of the distance from the optic disk to the margin. Three cells (small circles) within each of the outer eight zones were studied. The numbers indicate the percentage of the distance from the optic disk to the retinal margin. D, dorsal; N, nasal; O.D., optic disk.

Figure 7. Examples of computer-generated drawings of one cell from each of the eight concentric zones in the retina of Figure 6. The most peripheral zone is on the left, and cells to the right are from progressively more central zones. The axons of the cells are indicated with arrowheads pointing toward the optic disk.

of new retina peripherally. The angular subtense of the dendritic trees has been reduced because new peripheral retina was added as the retinal field remained constant (Easter et al., 1977). In the last part of this section, we will argue that the dendritic enlargement has kept pace with the retinal stretch.

Is the dendritic morphology dependent only on the age of the cell?

In all the comparisons made thus far, it was impossible to isolate the effects of age from those of general retinal enlargement. But in both small and large retinæ, there are cells of the same age; a comparison of their dendritic trees should reveal the effects that correlate with retinal size. We have used equation (3) to convert the distance from the optic disk into time (Fig. 10). (Because of the variability in the growth of the goldfish, the ages of the cells can only be roughly estimated; however, these estimates do allow useful comparisons to be made.) The cells occupying positions F and A in the small and large retinæ, respectively, are estimated to be about 6 months old. (No other pairs of positions could be equated for age.) These cells are at different relative distances from the optic disk and have qualitatively different dendritic morphologies, particularly with respect to the shape of the arbor (Fig. 10). Quantitatively, cells at the two locations are not so different; the numbers of branch points differ slightly, but the dendritic lengths and field sizes are very similar (Fig. 11). In summary, the qualitative appearance of the dendritic tree may well depend on factors such as the size of the retina and the location of the cell, but the total dendritic length and the area of the field appear to be age dependent.

Does the dendritic coverage remain constant with retinal expansion?

The coverage factor [planimetric density $\times$ mean area of the dendritic field (Brecha et al., 1984; Wässle et al., 1981a, b; 1983)] was determined for the subtype 1.2 cell in the central region of a small and a large retina, those of Figures 6 and 9, respectively. The locations of the cells were plotted by systematically scanning the retinæ at $625\times$ magnification and marking them on a camera lucida drawing, from which the planimetric densities were computed. They were 11.0 and 3.3 cells/mm² in the small and large retinæ, respectively. The average dendritic field areas, derived from Figures 8 and 11, were 0.16 and 0.56 mm², giving dendritic coverage factors of 1.76 and 1.85 for the small and large retinæ. This similarity suggests that the expansion of the dendritic fields has matched the expansion of the retina, as the drawing of a cell on a balloon would expand when the balloon is inflated.

Discussion

The proposed classification

We have divided ganglion cells into four types, 1–4 (Fig. 2), based on soma size and dendritic morphology as seen in the

whole-mount. These were subdivided into 15 subtypes based on their respective stratification patterns within the IPL and OPL (Fig. 4). When one imposes categories on a population of neurons, the aim is to create groups that have functional significance for the organism or structure being studied (Tyner, 1975). Conclusions as to the functional significance of the groups created, however, can only be reached after many characteristics, e.g., morphological, electrophysiological, and biochemical, are known. The only correlations available at the present time are electrophysiological. Dendrites ramifying within the outer

Figure 8. The average values of (A) number of dendritic branch points, (B) total dendritic length, and (C) dendritic field area, plotted as a function of distance from the optic disk for cells from the retina illustrated in Figures 6 and 7. Error bars; ± 1 SD.

Figure 9. Camera lucida drawings of the two retinæ used to study the changes in dendritic morphology with growth of the retina. At left is the retina from a young fish (ca. 1 year) and at right, that of an old fish (ca. 4 years). In both, the small circles give the locations of the cells studied. Within one retina, cells at positions labeled with the same letter lie at similar percentages of the distance from the optic disk to the margin. (The specific values are given in Figure 10.) Cells at positions C–G in the small retina are predicted to occupy positions C–G in the large retina. The dashed line on the large retina encloses the same number of cells that are central to positions C in the small retina. Cells at positions A and B in the large retina were added as the retina enlarged.

and inner thirds of the IPL lie within the electrophysiologically defined OFF and ON sublaminae, respectively (Famiglietti and Kolb, 1976; Famiglietti et al., 1977; Nelson et al., 1978) and can therefore be functionally classified. The response characteristics of cells with dendrites ramifying in the middle third are uncertain. With so little correlative data, the ganglion cell classification outlined in this paper is, as Rowe and Stone (1977) suggest, an hypothesis for categorizing them.

Ganglion cell morphology in other teleosts

The dendritic morphology of ganglion cells in several species of teleost fish has been described (Ito and Murakami, 1984; Kock, 1982b; Kock and Reuter, 1978b; Murakami and Shimoda, 1977; Naka and Carraway, 1975; Ramon y Cajal, 1972). Ramon y Cajal (1972) viewed cells in radial sections, thus making direct comparisons with cells viewed in whole mounts more difficult. Nonetheless, several of the typical teleostean ganglion cells that he described (see Ramon y Cajal, 1972; Plate 1, Fig. 6) can be recognized in the goldfish. Cells with large dendritic fields and thick dendrites that ramify within the inner or outer IPL were similar to our Type 1. Cells with small somata and thin dendrites in a small compact dendritic field were similar to our Type 2. In addition, Ramon y Cajal (1972) described cells that stratified within more than one level of the IPL, as did we.

Naka and Carraway (1975) described seven cell types, in the catfish, based on differences in the number and thickness of the primary dendrites. These seven appear to include the four types that we have distinguished in the goldfish, although Naka and Carraway did not subdivide theirs with respect to the level of stratification within the IPL.

Murakami and Shimoda (1977), using intracellular recording and staining of ganglion cells in the carp, described cells with large somata only, similar to our Type 1.

Kock and Reuter (1978b) and Kock (1982b) classified ganglion cells in the crucian carp and the goldfish by the size of the soma and dendritic tree and by the location of the soma. They concluded that there were three types: small cells with the soma in the ganglion cell layer, large cells with the soma similarly placed, and large cells with the soma displaced into the IPL. The small cells possessed small dendritic fields and were similar to our Type 2 cells. The large and large-displaced cells had large dendritic fields and were similar to our Type 1 cells. They did not distinguish cells with intermediate sizes or very sparse dendritic arbors and therefore probably included our Types 3 and 4 in the small-cell class. We did not find soma location useful in developing our classification, since we observed HRP-labeled somata of all four types displaced into the inner retina. The disagreement probably can be accounted for by the different methods; ours selectively labeled ganglion cells, while theirs (methylene blue and Golgi) did not.

Recently, Ito and Murakami (1984) have used a method similar to ours to label ganglion cells in two marine teleosts. Their cells were divided into six classes based on soma size and shape and size of the dendritic field. Cells with large somata and large dendritic fields (types V and VI) were similar to our Type 1. Cells with small somata and small dendritic fields (types I and II) resembled our Type 2. Their types III and IV were similar to our Types 3 and 4, respectively. In addition, Ito and Murakami (1984) showed that axonal diameters were positively correlated with soma size. Although we have not systematically studied this relationship, our impression is that it also obtains in the goldfish.

Several of the studies mentioned above also described the same general features of ganglion cell morphology that were seen in the goldfish. First, the somata of many ganglion cells were located on the optic disk side of the fan-shaped dendritic

Figure 10. Examples of computer-generated drawings of cells in the retinae of Figure 9. Those in the large retina are in the *upper row*; in the small retina, *lower row*. The percentage distance from the optic disk to the margin is the average value for all cells designated by the same letter; individual cells' distances were within 2% of this average. The estimated age of each cell in months is also given.

field, convex toward the margin (Kock, 1982b; Kock and Reuter, 1978b; Murakami and Shimoda, 1977). Second, the axon often originated from the proximal region of a major dendrite, sometimes far removed from the soma (Murakami and Shimoda, 1977; Naka and Carraway, 1975; see also Stell and Witkovsky, 1973, for descriptions of a cell in an elasmobranch). Third, many dendrites were webbed at branch points (Kock, 1982b; Stell and Witkovsky, 1973).

Not described in the studies above are the appendages present on the somata of some ganglion cells. The functional significance of these appendages is not known, although similar processes have been described on the immature neurons in the brain stem of the opossum and the cat (Morest, 1969). Neither have biplexiform ganglion cells (Type 3.4) been described before in fish. This may have resulted from their being infrequently stained by the methylene blue and Golgi impregnation techniques or from the problematic identification of ganglion cells (e.g., see Kock and Reuter, 1978b), especially those with processes in both plexiform layers.

The relative numbers of the various types remain uncertain. The Type 1 cells are very sparse, only about 1.3%. Our impression is that the Type 2 cells are the most abundant, but we cannot give a percentage. Displaced ganglion cells are quite rare, a few percent or less of HRP-labeled cells. Similarly, to judge from the most completely filled of retinal patches (Fig. 5), non-ganglion cell neuronal somata in the ganglion cell layer are also very rare.

Comparisons with mammals

The same qualitative features have been used to distinguish ganglion cell types in mammals, i.e., soma size, field size, density of arbors, and stratification. Types analogous to those in the

goldfish have been described (rat: Brown, 1965; Bunt, 1976; Perry, 1979; rabbit: Amthor et al., 1983, 1984; ground squirrel: West, 1976; West and Dowling, 1972; cat: Boycott and Wässle, 1974; Brown and Major, 1966; Kolb et al., 1981; Leventhal et al., 1980; Shkolnick-Yarros, 1971; Stone and Clarke, 1980; monkey: Boycott and Dowling, 1969; Perry and Cowey, 1984; Perry et al., 1984). The most obvious parallel is with the cat, whose alpha, beta, and gamma cell types, as described by Boycott and Wässle (1974) and Kolb et al. (1981), are qualitatively similar to our Types 1, 2, and 3, respectively. Although the functional classification of these morphological types is well established in cats, there is no corresponding functional classification in fish (see Bilotta and Abramov, 1985; Levine and Shefner, 1979).

Subtype 3.4 (Figs. 3, 4C) was the only one that ramified in the OPL (as well as the outer third of the IPL), and it therefore warrants the designation "biplexiform ganglion cell," as first described in the macaque (Mariani, 1982; Zrenner et al., 1983).

The somata of goldfish ganglion cells were usually displaced toward the optic disk within the dendritic field. In this respect, they differ from mammalian ganglion cells, whose somata are generally situated more centrally (Boycott and Wässle, 1974; Brown, 1965; Brown and Major, 1966; Kolb et al., 1981; Perry, 1979). This difference may be attributable to the very pronounced centropreperipheral gradient of neurogenesis and differentiation in fish. In mammals, this gradient is much less pronounced, and the processes of neurogenesis and differentiation are much less prolonged.

Functional correlates of dendritic growth

Four of the present observations bear on the ganglion cells' function during retinal growth. First, the sizes of the dendritic

Figure 11. Quantitative data for ganglion cells in the small and large retinæ. A, Number of dendritic branch points; B, total dendritic length; C, dendritic field area; and D, cross-sectional area of the soma, for cells in the small and large retinæ of Figures 9 and 10. Circles refer to individual cells, open circles in the small retina, filled circles in the large retina.

fields, measured in micrometers, increased. If, as is widely assumed, the extent of the dendritic tree determines the center of the receptive field, then the receptive field should have enlarged; this has been shown electrophysiologically (Macy and Easter, 1981). Second, estimates of optical parameters of the growing eye (Easter et al., 1977) indicate that, despite their physical growth, the angular subtense of dendritic fields decreases slightly. This has also been shown electrophysiologically for the receptive fields (Macy and Easter, 1981). Third, the coverage factor of the dendritic trees remained relatively constant with growth; this is a measure of the redundancy with which each point in the visual world is sampled. Its constancy was also suggested by electrophysiological results (Macy and Easter, 1981). Fourth, the thickening of the dendrites of the larger cells is probably functionally important, as it results in a larger space constant; that is, it enables the cells to integrate synaptic signals from the larger dendritic tree (Koch et al., 1982).

Determinants of dendritic growth in the retina

Wässle et al. (1981a, b; 1983) have shown that the dendritic fields of alpha cells in the cat overlap such that every retinal point is covered by at least one dendritic field, and that the ON- and OFF-center alpha cells do so independently. From these data, they inferred that cells of the same subtype interacted through their dendrites during development to regulate the extent of dendritic overlap, and thereby the size and shape of the dendritic fields.

The idea of exclusionary dendritic interactions has received experimental support. Linden and Perry (1982), Perry and Linden (1982), and Eysel et al. (1985) have shown that when a

patch of ganglion cells is destroyed in a young animal (rat in the first two studies, cat in the third), adjacent ganglion cells preferentially directed their dendrites toward this ganglion cell-free region. Similarly, Negishi et al. (1982) have shown that following pharmacological destruction of dopamine-containing cells (interplexiform cells?) in the goldfish, new cells subsequently produced at the margin directed their dendrites unusually long distances into the more central regions, free of dopamine-containing cells. These examples of abnormally directed growth are interpreted as having resulted from the absence of constraints normally present in the invaded region. Probably, the dendrites of all cells of a particular subtype repel one another. This repulsion may be expressed directly between dendrites or indirectly by competition for something in limited supply. [A similar hypothesis has been suggested to explain the restricted elaboration of the axon terminal fields of mechanosensory neurons in the leech (Kramer and Stent, 1985; Kramer et al., 1985).] The afferent input terminals and/or something they produce are possible candidates (Perry and Linden, 1982). Implicit in this interpretation is the idea that the dendrites of neighboring ganglion cells normally grow until the exclusionary forces between them have reached a steady state, at which time a characteristic dendritic overlap is achieved, and net dendritic growth ceases, because new dendrites cannot be maintained. Such interactions could account for the constant dendritic coverage of the retina by cells whose density varies regionally (Wässle et al., 1981).

Similar repulsive interactions could also account for much of the dendritic development in the peripheral retina of the goldfish. Here, most dendrites are oriented parallel to the margin, and centrally directed dendrites are relatively rare. This asym-

metry is probably attributable to dendritic interactions, as well, if one assumes that dendrites already in place have an advantage over new ones. We suggest that the first dendrites grow into the new retina, the annular zone adjacent to the margin, because it is virgin territory. Centrally directed dendrites encounter an area already occupied, and are therefore stunted. Later, as the retina enlarges by the addition of still newer tissue more peripherally, the erstwhile marginal cells send dendrites into it, and this combination of circumferential and centrifugal growth results in cells with fan-shaped dendritic trees, and cell bodies eccentrically positioned toward the optic disk (Fig. 1).

If local interdendritic interactions are supposed to determine dendritic development in peripheral retina, do they play a similar role more centrally, in that "plateau" region (Figs. 7, 10) where the dendritic arbors are all quite similar? It is impossible to answer this question with certainty, but none of our data requires such interactions. Indeed, the data are consistent with the idea that, once the lawn of mature dendrites has been established, subsequent enlargement of individual arbors may result from stretch (Hitchcock, 1985; Mastronarde et al., 1984). There are abundant examples elsewhere in the nervous system of the enlargement of a cell and its processes due to forces imposed from the outside. The spinal motoneuron, for instance, makes its initial embryonic contact with muscle only a few hundred micrometers away, but as the embryo enlarges, the axon stretches to many multiples of its original length. Bray (1984) has recently shown that elongation of neurites may be reproduced in cell culture by towing an initially short neurite with a slowly moving probe. We suggest that the dendritic enlargement in central retina—the enlargement illustrated at locations C, D, E, F, and G in Figure 10—may be attributed to the towing of the dendrites, perhaps as a result of hydraulic forces expanding the globe and stretching the retina (Coulombre, 1956).

Although this is the simplest explanation, there is another. The data are also consistent with the idea that dendrites are continually elongating and retracting (see Purves and Hadley, 1985); if so, the constancy of the numbers of branches noted in Figures 8 and 11 may reflect a much more active past, in which all dendrites at all times sought to enlarge but were limited by their repulsive neighbors.

The rates of dendritic elongation in the retina can be compared with rates of axonal elongation in these same cells, and neuritic elongation *in vitro* in other cells. The rate of axonal outgrowth in regeneration of the optic nerve is on the order of hundreds of micrometers a day (Murray and Grafstein, 1969). The *de novo* outgrowth of optic fibers in *Xenopus laevis* is even faster, about 1 mm/d (Holt, 1984). The neurites of dissociated chick sensory neurons in culture could be pulled at a rate of about 1 mm/d (Bray, 1984). In contrast, the dendritic elongation in the goldfish retina is very slow. The entire dendritic length of the cells at position B in Figure 11B is only about 9 mm, and these cells are estimated to be about 16 months old. Considering the rate of growth of all the dendrites, that works out to $9/548 = 0.016$ mm (16 μ m)/d. In central retina, where we have suggested that towing is a probable motive force, the rate is even lower. The total dendritic length of the cells at point G in the small retina (Fig. 11B) is about 5 mm. Over the course of 3 years, this increases to about 8 mm (solid symbols at point G in Fig. 11B). This works out to $(8-5)/1095 = 0.003$ mm (3 μ m)/d. It seems safe to conclude that the rate of dendritic elongation *in vivo* is not limited by any factor intrinsic to the cell; it must be limited by extrinsic factors, such as the interdendritic repulsion that we have hypothesized.

Summary and Conclusions

We have examined HRP-labeled ganglion cells in the goldfish retina and suggested a taxonomy, based on soma size and dendritic structure, that includes four major types, each of which

is further subdivided to yield 15 subtypes in all. One of these subtypes—a particularly large and sparsely distributed cell—has been examined in more detail, in an attempt to describe the stages, and to infer the determinants, of its development. We conclude that exclusionary interactions between dendrites of cells of this same subtype probably play an important role in the first few months of dendritic elaboration, and perhaps later, as well. When the mature dendritic arbor has been produced, subsequent growth proceeds without the net addition of new branches, and may result from stretch alone, analogous to the enlargement of a drawing on a balloon as the balloon is inflated.

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