Ganglion Cell Dendritic Structure and Retinal Topography in the Rat

J.D. SCHALL, V.H. PERRY, AND A.G. LEVENTHAL
Department of Anatomy, University of Utah School of Medicine,
Salt Lake City, Utah 84132 (J.D.S., A.G.L.); Department of Experimental Psychology,
Oxford OX1 3UD, England (V.H.P.)

ABSTRACT

The dendritic field size, the distribution of the dendrites relative to the cell body, and the overall shape of the dendritic field of type I ganglion cells in the rat retina were analyzed. These features of neuronal structure were related to the topography of the rat retina. As in the cat, the cell bodies of type I ganglion cells are arranged in a nonrandom mosaic. Previous work has demonstrated that the density of type I cells in the rat retina does not covary with the density of all ganglion cells. Type I dendritic field size varies over the retina; the increase in dendritic field size is accounted for better by the decrease in type I density than by the decrease in overall ganglion cell density. The center of the dendritic field of most type I cells is displaced in the plane of the retina from the cell body. Unlike in carnivore retina (Schall and Leventhal: J. Comp. Neurol. 257:149-159, '87), the dendritic fields in the rat are not displaced down the ganglion cell density gradient. Rather, there is a tendency for the dendritic trees, especially in temporal retina, to be displaced toward dorsal retina. Most of the dendritic fields are elongated, but the degree of elongation is less than that observed in carnivore or primate retina. Unlike in carnivore and primate retina (Leventhal and Schall: J. Comp. Neurol. 220:465-475, '83; Schall et al.: Brain Res. 368:18-23, '86), there is no relationship between dendritic tree orientation and position relative to any point on the retina in the rat. The foregoing differences in the morphology of retinal ganglion cell dendritic trees in rat, carnivore, and primate are considered in light of the differences in the development of retinal topography in these species.

Key words: rat retina, retinal ganglion, cell morphology, neuronal structure, retinal development
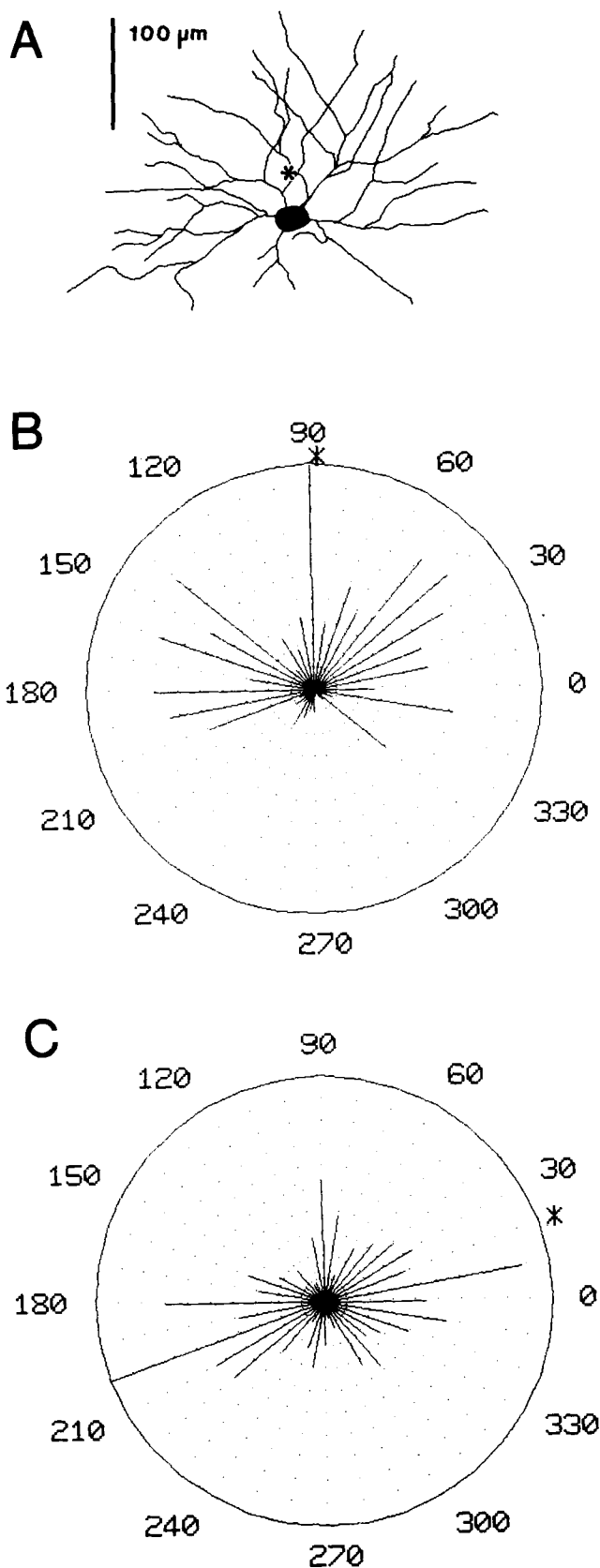
A well-defined ganglion cell density gradient is characteristic of cat (Hughes, '75; Stone, '78), ferret (Vitek et al., '85), and primate retina (Stone and Johnston, '81). Relationships between ganglion cell dendritic structure and retinal topography have been described in cat (Boycott and Wässle, '74; Kolb et al., '81; Leventhal and Schall, '83), ferret (Vitek et al., '85), and primate (Perry et al., '84; Rodieck et al., '85; Schall et al., '87). In the cat retina ganglion cell dendritic field centers are displaced from their cell body down the ganglion cell density gradient; further, the elongation and orientation of ganglion cell dendritic fields in the cat may be accounted for by the well-defined spatiotemporal pattern of retinal maturation in this species (Schall and Leventhal, '87).

We have analyzed the dendritic morphology and retinal topography of type I ganglion cells in the rat retina (Perry, '79); this class of cells exhibits the largest cell bodies and coarsest dendrites and may be analagous to the alpha cells observed in cat retina (Boycott and Wässle, '74). The purpose of this investigation was to examine (1) the distribution of the ganglion cell dendritic fields relative to their cell body in a retina with a very shallow ganglion cell density gradient (Lashley, '32; Fukuda, '77) and (2) the elongation and orientation of ganglion cell dendritic fields that develop in a retina that does not exhibit a well-defined spatiotemporal pattern of maturation (Morest, '70; Webster and Rowe, '85; Perry, unpublished results).

A

100 μm

B

90
120   60
150   30
180   0
210   330
240   300
270

C

90
120   60
150   30
180   0
210   330
240   300
270

MATERIALS AND METHODS

The dendritic trees of type I ganglion cells were visualized by using a neurofibrillar stain (Boycott and Peichl, '81). The density of presumed type I cells was assessed by counting all of the neurofibrillar stained cells in a 350 × 350-μm field at 0.5-mm intervals. The area centralis was defined as the region of maximum ganglion cell density. Camera lucida drawings of 226 ganglion cells from all regions of retina were traced into a computer for quantitative morphological analyses. These analyses have been described before (Leventhal and Schall, '83; Schall and Leventhal, '87) and will be only summarized here. Each dendritic tree was represented by a series of points; thicker, proximal trunks were represented by more points than were thin, distal twigs. The geometric centers of the cell body and of the dendritic field were determined. The distance separating the center of the dendritic field from the center of the cell body in the plane of the retina was measured. The direction of displacement of the center of the dendritic field from the center of the soma was measured relative to the dorsoventral axis of the retina.

The elongation and orientation of the dendritic fields were determined by calculating the mean vector of all the points representing the dendritic tree relative to the center of the dendritic field. The angle of the resultant vector gave the orientation of the dendritic field. The length of the resultant vector, termed the *orientation bias,* provided a measure of how elongated the dendritic field was. Orientation bias can range from 0 to 1 with 0 indicating a circular dendritic field. Figure 1 illustrates a computer representation of a type I ganglion cell and the morphological analyses. An explanation of the statistical tests used to analyze the distributions of angles may be found in Batschelet ('81).

RESULTS
Mosaic of ganglion cell bodies

To ascertain whether the type I ganglion cell bodies are arranged in a mosaic, the distance was measured between nearest-neighboring ganglion cells ramifying in laminae *a* (corresponding to OFF center cells) and *b* (ON center cells) of the inner plexiform layer in an 800 × 1,000-μm area of ventral temporal retina shown in Figure 2. The distribution of nearest-neighbor separations was compared to the distribution of separations of randomly distributed cells, which was calculated by using the equation derived by Wässle and Riemann ('78). The ratio of the mean to the standard deviation of the separations indicates how regularly spaced the cells are arranged (Wässle and Riemann, '78); this ratio will be referred to as the *mosaic ratio.* The average spacing between all type I cell bodies in the region sampled was 62

Fig. 1. **A:** Digitized computer representation of a type I ganglion cell in the rat retina. The 100-μm scale bar is aligned parallel to the dorsoventral axis of the retina. The asterisk is situated at the geometric center of the dendritic field. **B:** Circular histogram of the distribution of the dendritic field relative to the center of the cell body. Each line emanates from the coordinates of the center of the soma: the length of each line is proportional to the dendritic coverage in the given direction. The asterisk indicates the direction in which the center of the dendritic field is displaced from the center of the cell body. **C:** Circular histogram of the distribution of the dendritic field relative to the center of the dendritic field. The asterisk indicates the orientation of the dendritic field. Notice that this dendritic field is oriented horizontally, but the center of the dendritic field is displaced vertically from the cell body.
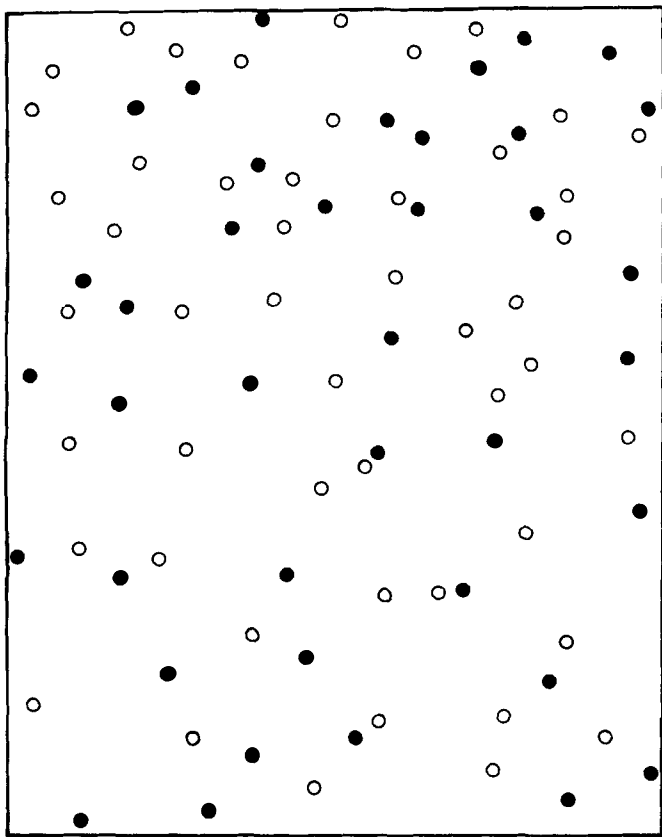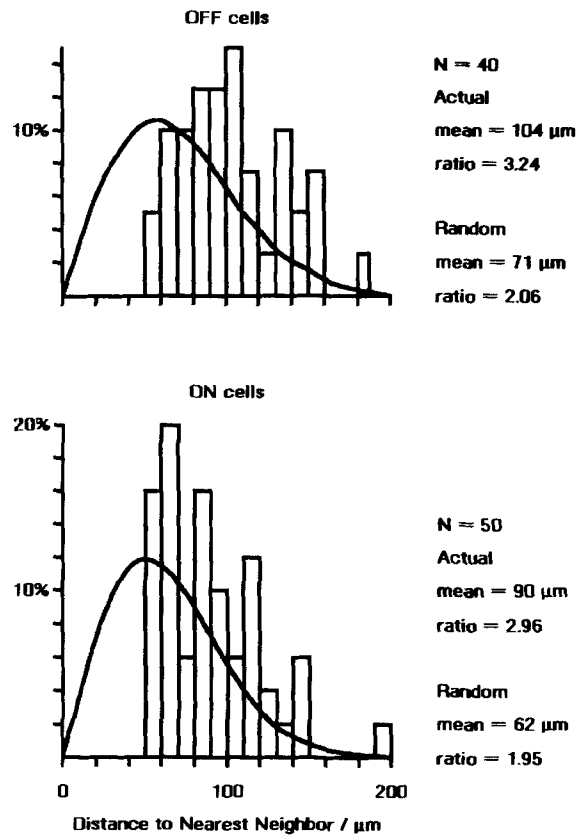
OFF cells

10%

N = 40
Actual
mean = 104 µm
ratio = 3.24

Random
mean = 71 µm
ratio = 2.06

ON cells

20%

10%

N = 50
Actual
mean = 90 µm
ratio = 2.96

Random
mean = 62 µm
ratio = 1.95

0   100   200

Distance to Nearest Neighbor / µm

Fig. 2. Arrangement of type I ganglion cell bodies in an 800 ×1,000-µm area of ventral temporal retina. The solid spots represent cells with dendritic field ramifying in sublamina a that are presumed OFF cells. Open spots represent cells with dendritic fields ramifying in sublamina b that are presumed ON cells. The distributions of separations of nearest-neighbor

OFF and ON cells are shown; superimposed on the histograms is the calculated spacing of a random distribution of cells of corresponding densities. The number of cells, mean separation, and mosaic ratio for the distributions are indicated. Ganglion cells in the rat retina are distributed in a nonrandom mosaic.

µm, and the mosaic ratio was 2.45. The average spacing of randomly distributed cells of the same density is 47 µm, and the mosaic ratio is 1.99. The observed and calculated random distributions were significantly different (Watson U^2 = 0.26, P <0.02). The average separation of ganglion cells ramifying in the b sublamina was 90 µm, and the mosaic ratio was 2.96. The average spacing of randomly distributed cells of the same density is 62 µm, and the mosaic ratio is 1.95. The observed and calculated random distributions were significantly different (U^2 = 0.23, P < 0.02). The average spacing between ganglion cells ramifying in the a sublamina was 104 µm, and the ratio was 3.24. The average spacing of randomly distributed cells of the same density is 71 µm, and the mosaic ratio is 2.06. The observed and calculated random distributions were significantly different (U^2 = 0.21, P < 0.05). These results indicate that type I ganglion cell bodies are distributed nonrandomly.

Variation in dendritic field size

The diameter of type I ganglion cell dendritic fields varies over rat retina. In the rat retina the density of type I ganglion cells does not covary with the density of all ganglion cells; rather, the point of peak type I density lies temporal to the area centralis (Dreher et al., '85; Reese and

Dendrites Field Diameter/µm

300

250

200

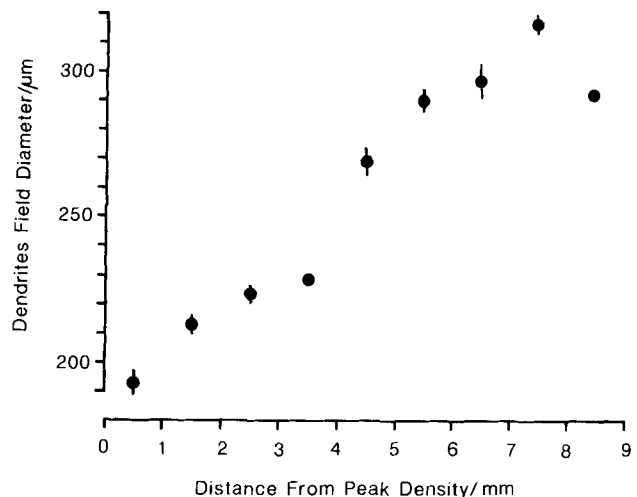0   1   2   3   4   5   6   7   8   9

Distance From Peak Density/ mm

Fig. 3. Type I dendritic field diameter as a function of distance from the point of peak type I density. Each spot represents the mean diameter of the dendritic fields in 1-mm increments. The vertical bars represent the standard error; in some cases the standard error was smaller than the size of the symbol. Dendritic field diameter increases with the distance from the point of the peak type I density.

Cowey, '86). Type I dendritic field diameter is plotted vs. distance from the point of peak type I ganglion cell density in Figure 3. Dendritic field size increases as type I ganglion cell density decreases. The increase is relatively small, compared to what is observed in other species, as the largest type I dendritic fields are only 50% larger than the smallest.

The change in type I cell dendritic field diameter was also related to distance from the position of the area centralis. The change in dendritic field diameter was accounted for better by distance from the point of peak type I density ($r^2 = 0.50$) than by distance from the area centralis ($r^2 = 0.42$). The dendritic fields in the region of the peak type I density in two retinae, with mean diameters of 214 $\pm$ 3.2 μm and 162 $\pm$ 3.6 μm, were significantly smaller than the dendritic fields in the area centralis, with means of 229 $\pm$ 6.8 μm and 175 $\pm$ 2.8 μm (t = 2.2, df = 20, $P < 0.05$ and t = 2.9, df = 39, $P < 0.01$). These results indicate that the change in the dendritic field size of type I cells is related to the change in type I density and not to the change in overall ganglion cell density.

Distribution of the dendrites relative to the soma

The magnitude of displacement of the center of the dendritic field from the center of the cell body for all of the type I cells is shown in Figure 4. There is a significant tendency for the center of the dendritic fields to be displaced from the center of the cell bodies. The average dendritic displacement of the sampled cells was 35 μm.

In Figure 5 the mean magnitude and direction of displacement of the dendritic fields in 1-mm^2 areas of one retina are shown. The magnitude of dendritic displacement tends to be slightly greater in temporal (average of 38 μm) than in nasal (32 μm) retina. There is no statistically significant tendency for the dendritic fields to be displaced down the very shallow ganglion cell density gradient. However, there is a significant tendency for the dendritic fields to be displaced dorsally (for all cells, z = 43.97, $P < 0.001$). The

Fig. 5. Magnitude and direction of displacement of type I dendritic trees in one retina. The angle of each arrow indicates the mean direction of displacement of cells in 1-mm^2 areas of retina. The length of each line relative to the 50-μm scale is proportional to the average magnitude of displacement. The open spots indicate areas containing just one ganglion cell. The contours represent type I density values (cells/mm^2) as follows: a, 175; b, 125; c, 100; d, 50. Notice that the dendritic fields are not displaced down the shallow density gradient; rather, there is a tendency for dorsal displacement that is more pronounced in temporal than in nasal retina.

dorsal displacement tendency is more pronounced in temporal (z = 42.61, $P < 0.001$) than in nasal (z = 6.68, $P < 0.001$) retina.

Elongation and orientation of the dendritic trees

Figure 6 shows the distribution of orientation biases for all of the sampled type I dendritic fields. The average orientation bias was 0.19, which corresponds to a ratio of the length of the longest to the length of the orthogonal axis of the dendritic trees of approximately 1.4. Previous work in
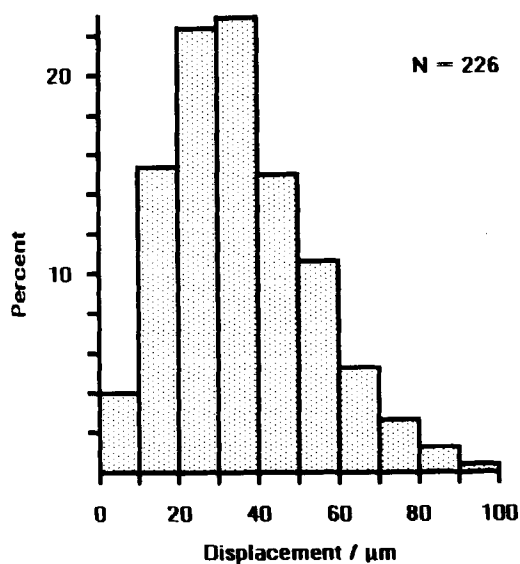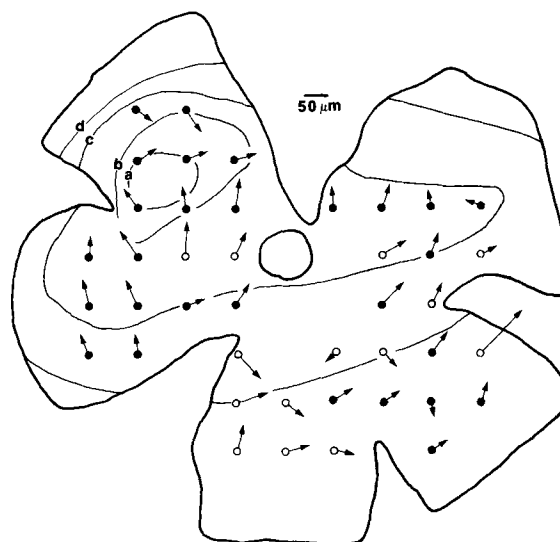

Fig. 4. Displacement of type I ganglion cell dendritic fields from their somas. The distance was measured between the center of the dendritic field and the center of the soma in the plane of the retina. The dendritic field of most type I ganglion cells is displaced from the soma. The average displacement of the sample is 35 μm.
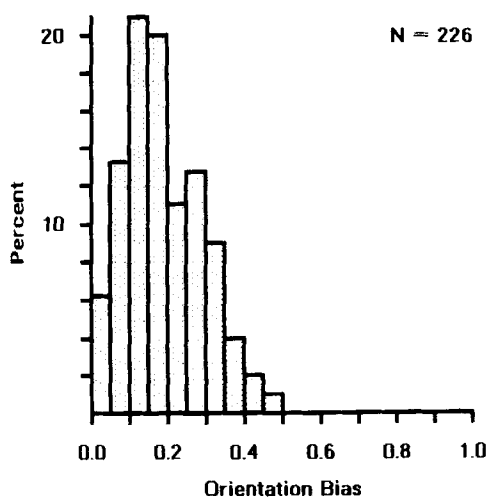

Fig. 6. Orientation biases of type I ganglion cell dendritic fields. Orientation bias measures the elongation of the dendritic tree. The values range from 0 to 1; a circular dendritic tree has an orientation bias of 0. The mean orientation bias of the type I dendritic trees sampled is 0.19, and 81% of the dendritic fields exhibit an orientation bias greater than 0.1.
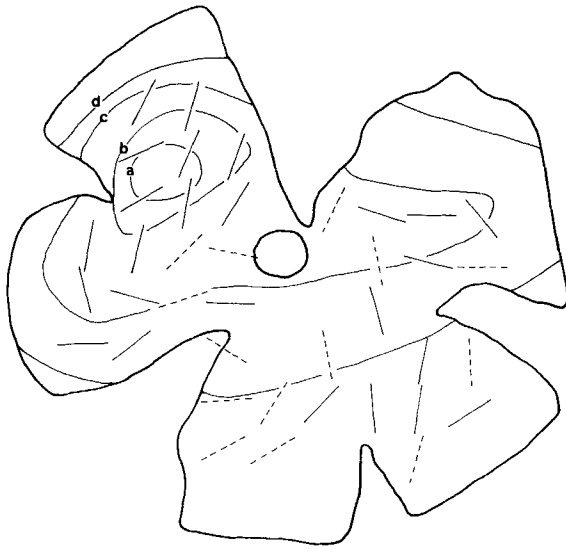
Fig. 7. Type I ganglion cell dendritic field orientation in one retina. Each line is inclined along the mean orientation of the significantly elongated dendritic trees in each 1-mm² area of retina. The dashed lines indicate areas with just one elongated dendritic field. The isodensity contours are as in Figure 5. Notice that there is no correlation between dendritic field orientation and retinal position.

the cat has indicated that an orientation bias of 0.1 represents a significant degree of elongation (Levick and Thibos, '82; Leventhal and Schall, '83). According to this criterion 81% of the type I dendritic trees sampled were significantly elongated.

In Figure 7 is shown the mean orientation of the significantly elongated dendritic fields in 1-mm² areas of one retina. There is no overall anisotropy in the distribution of dendritic field orientations of all the cells (z = 2.42, P > 0.5). To ascertain whether there is any relation between dendritic field orientation and position, the difference was measured between the orientation of each elongated dendritic field and the angle connecting the cell to different points on the retina. If the dendritic fields were oriented radially with respect to a point, then the angle differences would cluster at 0°. The type I dendritic fields are not oriented radially with respect to the area centralis (u = 1.35, P > 0.5), nor relative to the point of peak type I cell density (u = 0.57, P > 0.5), nor relative to the optic disk (u = 2.29, P > 0.5).

DISCUSSION

The present investigation has demonstrated the following: (1) type I ganglion cell bodies are arranged in a nonrandom mosaic; (2) type I dendritic field size increases as type I density decreases, and the change in type I dendritic size is not accounted for as well by the change in overall ganglion cell density; (3) the center of the dendritic field of type I ganglion cells tends to be displaced laterally from the cell body, but the dendritic fields do not tend to be displaced down the type I ganglion cell density gradient; instead, there is an apparent tendency to be displaced dorsally; (4) type I ganglion cell dendritic fields are only slightly elongated and are not systematically oriented relative to any point on the retina.

Dreher et al. ('85) related type I ganglion cell size to the density of all ganglion cells. They demonstrated that type I

soma and dendritic field size increased as total ganglion cell density decreased. Since the density of type I cells does not covary with the density of all ganglion cells (Dreher et al., '85; Reese and Cowey, '86), the present investigation related type I dendritic field size to the density of type I cells. The variation in dendritic field size was accounted for best by the change in type I cell density. This result is consistent with the hypothesis that class-specific dendritic interactions govern dendritic field size.

Interactions between ganglion cell dendrites have been hypothesized in the cat based on the mosaic arrangement of ganglion cell bodies (Wässle and Riemann, '78). Experimental evidence for competition between ganglion cell dendrites in cat retina also has been provided; if the ganglion cells in a spot of retina are eliminated in neonatal cat retina, the dendrites of ganglion cells surviving on the border grow into the depleted region (Eysel et al., '85; Ault et al., '85; Leventhal et al., '87). Also, ganglion cells that develop in areas of experimentally reduced density are significantly larger than normal (Ault et al., '85; Leventhal et al., '86). Displacement of ganglion cell dendrites down the ganglion cell density gradient is also evident in the normal adult cat retina (Schall and Leventhal, '87) as well as in the ferret (unpublished observations).

There is also evidence for dendritic competition between ganglion cells in the rat retina. This investigation has demonstrated that the cell bodies of type I ganglion cells in a spot of retina are arranged in a mosaic. Furthermore, if the ganglion cells in a spot of neonatal rat retina are eliminated, the dendrites of surviving ganglion cells on the border extend into the evacuated region (Linden and Perry, '82; Perry and Linden, '82). The dendritic fields of type I ganglion cells in the rat retina, however, do not tend to be displaced down the shallow density gradient. Instead, there is a tendency for the dendritic fields of type I ganglion cells, especially in temporal retina, to be displaced dorsally. It may be possible to reconcile the lack of dendritic displacement down the density gradient with the other evidence for dendritic interactions by postulating that the normal ganglion cell gradient in rat retina is too shallow to provide any directional guidance for the growing dendrites. In rat retina significant changes in density occur over millimeters, not microns, as in the cat and monkey. While we can only speculate why the dendritic fields are displaced dorsally, it is noteworthy that dorsoventral asymmetries in retinal development have been described (Mann, '28).

Dreher et al. ('85) also investigated the elongation and orientation of type I ganglion cell dendritic fields. Measuring the elongation as a ratio of the length of the long axis to the length of the short axis of the dendritic tree, they report that 65% of the type I cells sampled had ratios greater than or equal to 1.2. Using a more sensitive computer analysis we find that the mean orientation bias was 0.19 and 81% of the dendritic fields were significantly elongated according to previously defined, functional criteria (Levick and Thibos, '82; Leventhal and Schall, '83). In the cat 88% of the ganglion cell dendritic fields are significantly elongated, and the mean orientation bias is 0.26 (Leventhal and Schall, '83). In the ferret 85% of the dendritic fields are significantly elongated, and the mean orientation bias is 0.25 (Vitek et al., '85). In the macaque 88% of the dendritic fields are significantly elongated, and the mean orientation bias is 0.27 (Schall et al., '86). Hence, ganglion cell dendritic fields in the rat are less elongated than their counterparts in carnivores and primates.

Dreher et al. report that there is no correlation between dendritic field orientation and the angle of the cell relative to the area centralis. The present results support this finding. Dreher et al. do, however, report that there is a weak correlation between dendritic orientation and the angle to the optic disk. This was not found in the present study, and subjecting the published data of Dreher et al. to the V test (Batschelet, '81) indicates that actually there is also no statistically significant tendency in their sample (u = 1.64, P > 0.5). In this report evidence is also presented that type I cell dendritic fields are not oriented relative to the point of peak type I cell density.

Retinal ganglion cell dendritic fields are oriented approximately radially relative to the area centralis in the cat (Leventhal and Schall, '83) and ferret (Vitek et al., '85) or to the fovea in the macaque (Schall et al., '86) and human (Rodieck et al., '85). The fact that ganglion cells in the rat retina are less elongated and that their orientation is unrelated to retinal position indicates that the process by which ganglion cell dendritic fields become elongated and oriented is weaker in the rat than in carnivores and primates.

Carnivore and primate retinae are distinguished from the poorly specialized rodent retina by the presence of well-defined retinal topography. The topography of the retina appears to arise from the pattern of retinal development (reviewed by Rapaport and Stone, '84). In the cat there is a significant correlation between the elongation and orientation of ganglion cell dendritic trees and the geometry of a wave of maturation that passes over the retina from embryonic day 50 to postnatal day 10 (Schall and Leventhal, '87). While there is a centrifugal gradient of development in the rat retina, the pattern of maturation is not as well defined spatiotemporally as that in the cat (Morest, '70; Webster and Rowe, '85; Perry, unpublished observations). The fact that the ganglion cell dendritic fields in the rat are less elongated and not systematically oriented is consistent with the hypothesis that the elongation and orientation of dendritic fields are related in an as-yet-unknown fashion to the wave of maturation.

ACKNOWLEDGMENTS

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