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Factors controlling the dendritic arborization of retinal ganglion cells

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Abstract

The effects of changing retinal ganglion cell (RGC) density and availability of presynaptic sites on the development of RGC dendritic arbor in the developing chick retina were contrasted. Visual form deprivation was used to induce ocular enlargement and expanded retinal area resulting in a 20–30% decrease in RGC density. In these retinas, RGC dendritic arbors increased in a compensatory manner to maintain the inner nuclear layer to RGC convergence ratio in a way that is consistent with simple stretching; RGC dendritic arbors become larger with increased branch lengths, but without change in the total number of branches. In the second manipulation, partial optic nerve section was used to produce areas of RGC depletion of approximately 60% in the central retina. This reduction in density is comparable to the density of locations in the normal peripheral retina. In RGC-depleted retinas, dendritic arbor areas of RGCs in the central retina grow to match the size of normal peripheral arbors. In contrast to the expanded case, two measures of intrinsic arbor structure are changed in RGC-depleted retinas; the branch density of RGC dendrites is greater, and the relative areas of the two arbors of bistratified cells are altered. We discuss the potential roles of retinal growth, local RGC density, and availability of presynaptic terminals in the developmental control of RGC dendritic arbor.

Keywords: Chicks, Dendritic arborization, Optic nerve section, Retinal development, Visual deprivation

Introduction

The divergence and convergence of connectivity from photoreceptors to inner nuclear layer cells to retinal ganglion cells underlies the functional variation in the analysis of visual information. Differences in the convergence ratio of retinal neurons underlie scotopic or photopic vision, and high or low spatial resolution. These differences in convergence are observed in different zones of the retina having different functions (such as the retinal center and periphery) and between species with different retinal organization (such as those specialized for panoramic vision versus those for high acuity in the central visual field). Despite the importance of the retinal convergence ratio to visual function, little is known about the mechanisms that establish and regulate it.

Much effort has been spent on the description and classification of anatomical and functional cell types in the adult retina. By functional classes, we refer to either physiological distinctions (such as ON- vs. OFF-center responses, chromatic opponency, or directional selectivity), or anatomical distinctions (such as arbor stratification, size, or complexity). The development of these functional classes is less well understood. It is unknown, for instance, whether different functional classes of retinal ganglion cells possess intrinsically different developmental mechanisms for recognition and organization of their input, or whether adult variation is simply the result of a small number of developmental mechanisms expressed in various cellular environments. In this paper, we examine the control of retinal neuron growth with respect to the particular case of the dendritic arbors of retinal ganglion cells (RGCs), the best studied component of the developing retina.

One possible source of variation in dendritic structure is the intrinsic genetic instructions for process extension. The rate of growth, frequency of branching, and angle of branching may vary according to cell lineage and remain independent from interaction with the immediate cell environment. In support of this view, isolated ganglion cells in culture show a stereotyped rate of growth and branching pattern distinct from neurons from other regions in the central nervous system, and re-express branching patterns similar to their pattern shown *in vivo* (Montague & Friedlander, 1989, 1991). Transplanted RGCs without central targets show relatively normal morphological maturation (Sakaguchi, 1989).

Intraretinal factors are, however, clearly involved in the control of dendritic form and size within retinal ganglion cell classes. RGC dendritic arbor size changes with eccentricity (Wässle et al., 1981*a*,*b*; Schall & Leventhal, 1987) and may scale proportionately with retinal growth (Hitchcock & Easter, 1986; Bloomfield & Hitchcock, 1991). Experimental increases or decreases

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of RGC density during development change RGC arbors in the compensatory direction (Linden & Perry, 1982; Eysel et al., 1985; Kirby & Chalupa, 1986; Leventhal et al., 1988; Perry, 1989). Density-related interactions can occur both between like and unlike morphological cell types (Deplano et al., 1994).

Cell activity is also involved in dendritic development. Reduction of ganglion cell spiking activity slightly reduces dendritic length and complexity (Wong et al., 1991). Bodnarenko and Chalupa (1993) report that stratification of retinal ganglion cell dendrites in the inner plexiform layer, one of the mechanisms segregating functional channels in the retina, is prevented by eliminating ON-center bipolar cell activity in the developing retina.

Complex interactions of both genetic and environmental factors also determine RGC type and structure as has been shown in the differentiation of retinal cell types (e.g. Adler, 1993; Cepko, 1993). In the cat, alpha and beta RGC somas and arbors vary in size with local cell density, either in normal center-to-periphery or experimentally induced density variation, but their basic branching structure and relative size persists in all environments examined (Kolb et al., 1981; Kirby & Chalupa, 1986; Leventhal et al., 1988). At different times of development, and for different cell classes, the fundamental nature of arbor extension changes, resulting in progressive alteration of coverage factors and dendritic complexity (Maslim et al., 1986; Ramoa et al., 1988; Deich et al., 1994). The growth program employed by a cell's dendrites could also be selected or instructed by the target interactions of a cell (Wingate & Thompson, 1994).

Thus, several epigenetic factors controlling RGC development and dendritic growth are possible: competition for available synaptic input, availability of space for growth, contact inhibition with other cells of similar or dissimilar type, direct mechanical transduction of the forces of eye growth to membrane extension, and trophic responses mediated by retinal activity. In normal retinal growth these factors, however, often covary. For example, in the retinal periphery, RGC competitors have a low spatial density, and they also have increased convergence of inner nuclear cells providing greater synaptic input.

In the present study, we contrast the effects of two possible sources of control of RGC dendritic arborization. First, we manipulated retinal size to reduce RGC density along with the density of all other cell classes. In several species, visual form deprivation results in ocular enlargement and increased retinal area (e.g. Raviola & Wiesel, 1985; Wallman & Adams, 1987; Norton, 1990; Troilo & Judge, 1993). Increased retinal area reduces the density of all populations of cells in the retina, but the ratio of ganglion cells to bipolars and amacrines does not change. Alternatively, we reduced RGC density by partial optic nerve section. Partial optic nerve section also reduces RGC density, but with an increased ratio of afferent-to-ganglion cells that offers greater-than-normal convergence and availability of synaptic sites. This contrast allows us to separate the effects of density of neighboring ganglion cells from the effects of presynaptic input on ganglion cell dendritic size and arbor complexity. Preliminary reports of this investigation have been given (Troilo et al., 1994*a*,*b*).

Methods

Subjects

Twenty-four white leghorn chicks (Cornell-K strain) were used in this study. The chicks were reared until the age of 4 weeks in temperature controlled brooders under a 12-h diurnal cycle. Food and water were available *ad libitum*.

Experimental manipulations

Chicks were raised with two different manipulations to alter the density of RGCs. Visual form deprivation was used to produce ocular enlargement and retinal expansion (expanded). This procedure is not known to alter neuron numbers in the inner nuclear layer and the RGC layer. Seventeen chicks were raised with full field visual deprivation of one eye using white translucent occluders (after Wallman et al., 1978). At 1–2 days of age, the occluder devices were attached to the feathers surrounding the experimental eye using Collodion (Fisher Scientific, Pittsburgh, PA) and left in place until 4 weeks of age. This is a period of rapid eye growth in the chick and visual deprivation at this time produces significant ocular enlargement (Wallman & Adams, 1987) and an increase in retinal area (Troilo et al., 1994*a*,*b*).

In seven other chicks, partial optic nerve section was used to deplete RGCs across the retina (depleted) without retinal expansion. Three-day-old birds were anesthetized with a mixture of pentobarbital (2.4 mg/100 g) and chloral hydrate (10 mg/100 g). Using a dissecting scope the optic nerve just behind the globe was exposed as described by Troilo et al. (1987). The epineurium was cut and, using a fine pair of forceps, small groups of the optic nerve fibers were removed from various depths within the nerve. In both groups the contralateral eye was untreated and served as control. At 4 weeks of age, the axial lengths of both eyes of all chicks were measured with A-scan ultrasound biometry. Since depletion of RGC's is not thought to change inner nuclear cell number (see Finlay, 1992), the intent of this manipulation is to increase the convergence ratio between the inner nuclear layer and the retinal ganglion cell layer during the developmental period identical to the expansion case described above. In both the expansion case and the depletion case, stability or alteration of RGC number was verified as described below. Studies of any resultant changes in inner nuclear cell number or conformation are in progress (Crowley et al., 1995; Xiong et al., 1995).

Retinal ganglion cell anatomy

At 4 weeks of age all chicks were euthanized with sodium pentobarbital (50 mg/ml, 0.7 ml, i.p.). The eyes were immediately enucleated and equatorial and axial dimensions were measured with calipers. The anterior segments were removed and the posterior segments were postfixed for 5 min in 4% paraformaldehyde and prepared for anatomical analysis. The retinas were eventually dissected free from the posterior segments and the retinal pigment epithelium was removed. The retinas were then prepared as whole mounts. Total retinal area was determined in every subject for both eyes and paired comparisons were made between the experimental retina and the contralateral control retina.

The retinas were processed for either Nissl staining of the ganglion cell layer or labeling of the ganglion cell dendrites. To determine the change of RGC number and density distribution in the expanded retinas, six retinas (three control and three expanded) were prepared as whole mounts shortly after enucleation. The retinas were mounted on slides with the RGC fiber layer up and were stained with cresyl violet. All cells in the ganglion cell layer were counted using a computer-assisted camera-lucida system. The retina was sampled in a regular grid every

2.5 mm along the horizontal and 0.6 mm along the vertical meridian, giving about 200 samples per retina. We included in our counts all cells in the retinal ganglion cell layer including displaced amacrines and glia (estimated to be 35% of the total population by Ehrlich, 1981).

RGC dendritic arbor anatomy was visualized in individual cells from both the expanded and depleted retinas, and their paired controls, using the carbocyanine dye DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, Molecular Probes, Eugene, OR; see Godement et al., 1987). After enucleation and removal of the anterior segment, a small amount of DiI was inserted into various positions in the optic nerve head with a fine stainless-steel wire. In the depleted retinas, it was desirable to place more DiI into the optic nerve head to insure that a sufficient sample of ganglion cells were labeled. In these cases a fine forceps was used to place a piece of DiI into the nerve head. The eyes were then placed into 4% paraformaldehyde and stored in darkness for between 4 and 14 weeks. Different incubation times were used to transport dye different distances along cell membranes so that we could sample cells at various eccentricities from the optic nerve head. The retinas were subsequently removed, prepared as whole mounts, and placed on slides for fluorescence microscopy.

Examples of DiI-labeled cells are shown in Fig. 1. A total of 347 cells from 33 retinas was examined. DiI-labeled cells were collected for analysis if their dendritic arbors were isolated from those of neighboring cells. In the control group (combining the paired control eyes from both expanded and depleted experimental subjects), 127 cells were sampled from 13 retinas. In the expanded group, 13 retinas were examined and 116 cells were sampled. In the depleted group, seven retinas were examined and 104 cells were sampled. The distribution of sampled cells across the retina was similar for control, expanded, and depleted retinas (Fig. 2). Cells were grouped into central or peripheral retina divisions based on their position relative to the 14,000 cells/mm^2 isodensity contour. In the depleted group, ganglion cell density in the area (180 × 241 μm) surrounding a DiI-labeled cell was determined by counter-staining for 40–60 min with a 0.02% buffered solution of the fluorescent dye bisbenzamide (Sigma Chemical Co., St. Louis, MO). The degree of ganglion cell density reduction in a particular retinal location in the optic nerve sectioned eyes was calculated (after adjusting for glia and displaced amacrine cells) by comparing the bisbenzamine label density with the density of control eyes at comparable locations. Cells selected for sampling, on the criteria described previously, were in regions of density reduction of at least 20% (mean 63%).

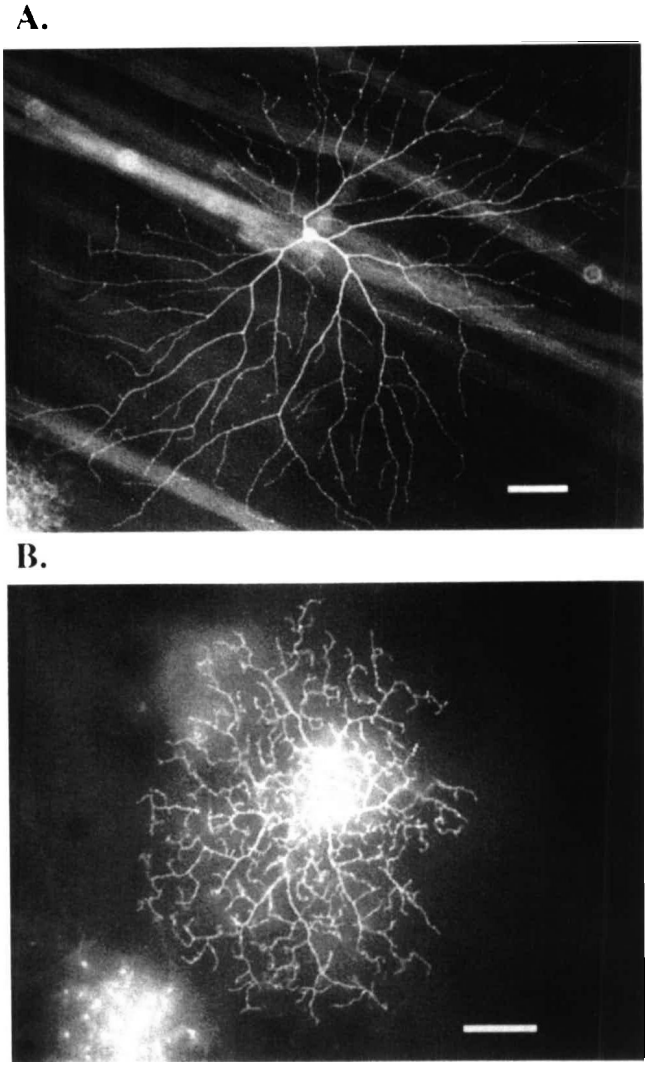

Fig. 1. Examples of DiI-labeled chick retinal ganglion cells. (A) Alpha cell. Scale bar = 50 μm. (B) Beta cell. Scale bar = 25 μm.

Morphometry

For all cells, RGC arbor dimensions were measured using video image analysis (NIH Image) on a Macintosh computer equipped with a video capture board (Data Translations, Marlboro, MA). To produce an image of each DiI-labeled cell, approximately 30 images were captured and averaged to increase contrast and reduce background noise. For multistratified cells, we measured the arbor area at each stratum, and except when mentioned, the area of the larger proximal strata was used for overall comparisons between treatment groups. The minimum and maximum diameters and area of the arbor field were measured for each cell.

In a subset of cells that had high enough isolation and resolution of their total dendritic branches, we further analyzed arbor structure by measuring total arbor length, mean branch length, number of branches, and branches per unit area. In all treatment groups, such isolatable cells were consistently larger in arbor area than the mean of all cells measured, in any retinal location, although all fell in our beta-cell criterion range (see Fig. 3, data relating to beta cell criterion; Fig. 4, arbor sizes of isolatable cells; and Fig. 5, arbor sizes of all measured cells). Fifteen cells from normal retinas, 19 from expanded retinas, and 23 from depleted retinas were measured in this fashion.

The data were analyzed by analysis of variance and regression with two computerized statistical packages; SuperANOVA and SYSTAT.

Results

Retinal ganglion cell properties in the normal chick retina

Cell classes

Our analysis of RGCs in the chick retina is consistent with an earlier analysis by Ehrlich (1981) and suggests that there are two major categories which resemble the alpha and beta cells of the cat retina. Fig. 1 shows examples of the alpha and beta retinal ganglion cell types identified in chicks. We used a crite-

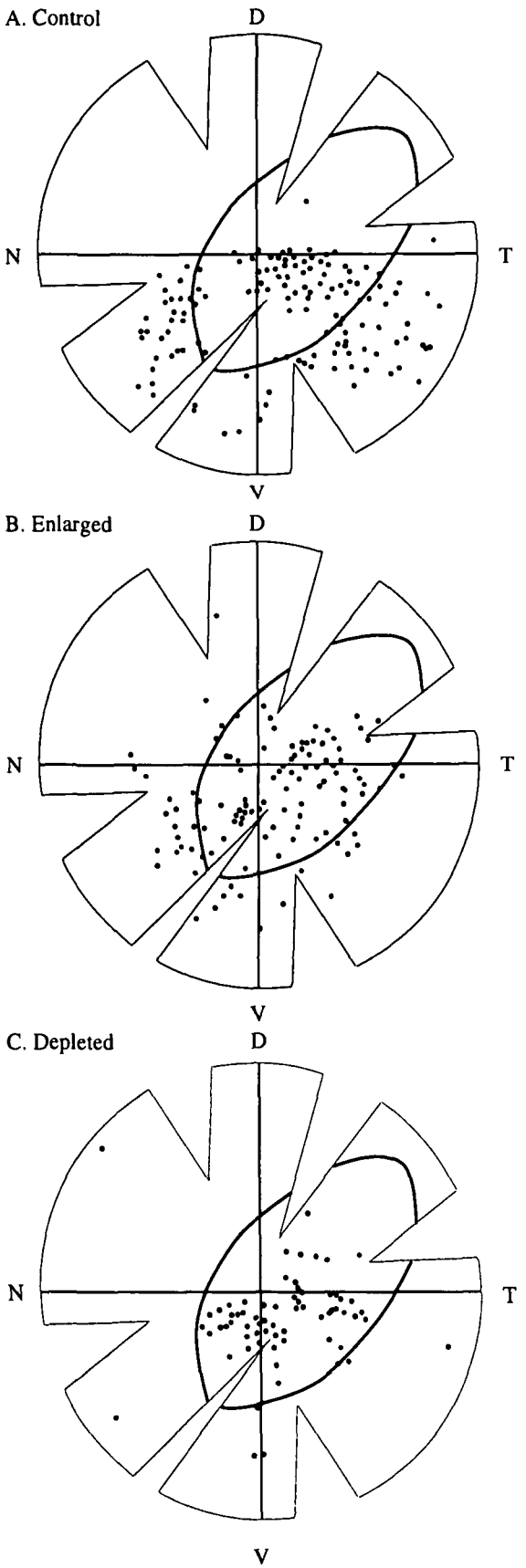

Fig. 2. Schematic drawings of normalized retinal whole mounts with an isodensity contour (14,000 cells/ mm^2) delineating the central and the peripheral retina. The distribution of sampled cells across the retina is similar for control, expanded, and depleted retinas. Individual points represent the locations of sampled cells. (D: dorsal; V: ventral; N: nasal; and T: temporal)

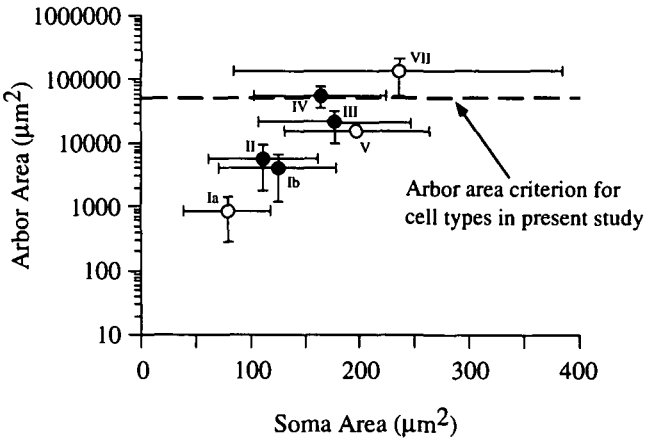

Fig. 3. Soma size and arbor area of several types of chick retinal ganglion cells described by Thanos et al. (1992). The dashed line represents the cutoff size criterion (50,000 μm^2) for alpha and beta cells in our sample. Data points represent the mean ± S.D. of soma and arbor areas for cell categories as described by Thanos et al. Cell categories Ib, II, III, and IV (filled circles) correspond to beta cells in our sample. The unfilled circles represent categories Ia (small, polymorphic, sparsely branching), V (large sparsely branching cells with eccentrically placed nuclei, and VII (similar to alpha cells in this study).

rion of 50,000 μm^2 arbor area as a cutoff between the alpha and beta categories (Figs. 3, 4, and 5A). Beta cells had smaller somas (Figs. 4A, and 5A), and less arbor (Fig. 4B). Thanos et al. (1992) have previously described seven RGC classes in the chick. Our beta and alpha categories conform to Thanos et al.'s classes Ib-IV and VII, respectively . As is shown in Fig. 3, classes Ib-IV differ only in size and not in their structural description. Arbor density of class Ib-IV cells is greater than in the larger class VII cells (Fig. 4C). The mean branch angle of those cell classes corresponding to our beta class is approximately 90 deg, while the branch angles of class VII (alpha cells) cluster around 60 deg (see the branch angles in Fig. 1). The remaining cell classes account for less than 10% of the population: class Ia (about 2%) are small polymorphic cells with few branches; class V (7%) are bottle-shaped, sparsely branching cells with eccentrically placed cell bodies (Thanos et al., 1992; Snow et al., 1994). We present data only from cells with arbors smaller than 50,000 μm^2 (Fig. 5A), which made up our largest sample (295 cells with area measured, 57 cells with arbor structure analysis). Some small, sparsely branching, non-beta cells will be included in this analysis by this criterion, but only as a small fraction.

Cell distribution

The density distribution of cells in the RGC layer (both retinal ganglion cells and displaced amacrine cells) in the chick retina normally varies with eccentricity. Fig. 6 (top) shows examples of Nissl-stained cells located in central and mid-peripheral (60–120 deg eccentricity) control retinas. There is a significant reduction in RGC layer cell density from central retina toward the periphery (ANOVA, $P < 0.01$). The mean (±S.D.) RGC layer cell density in the central 60 deg of retina is 16,806 ± 1674 cells/mm^2. The density is reduced to 12,310 ± 1140 cells/mm^2 in the mid-peripheral retina, and 8111 ± 65 cells/mm^2 in far-peripheral retina (>120-deg eccentricity). For the rest of our analyses, when relevant, we grouped mid-peripheral and far-peripheral into a single "peripheral" group.

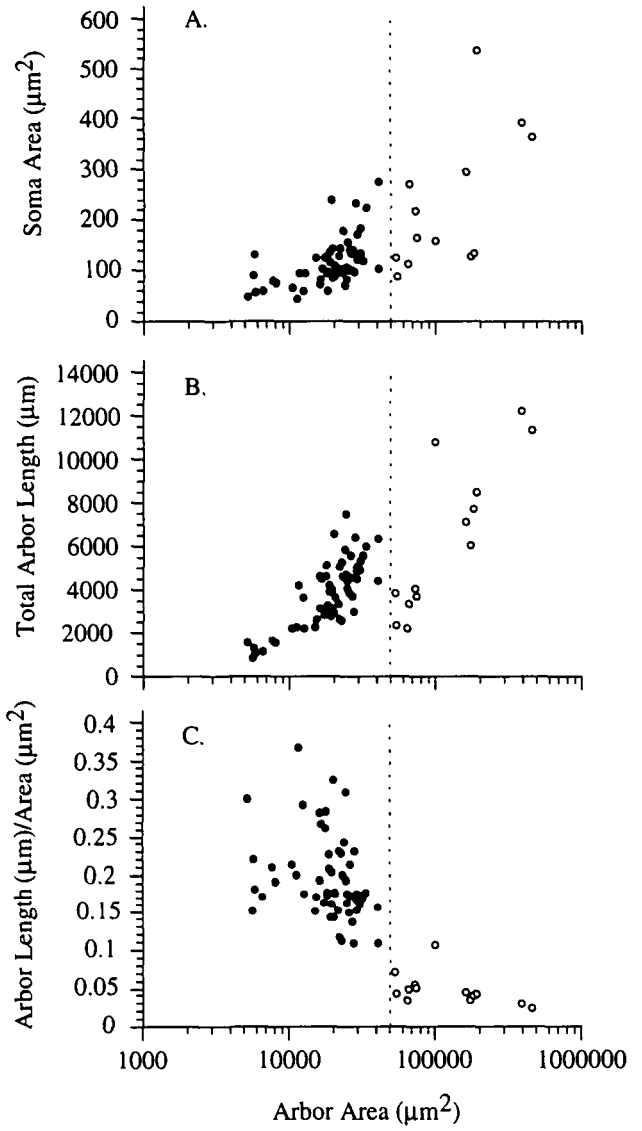

Fig. 4. The relationship of arbor area to soma area, total arbor length, and arbor length per unit area (arbor density). The cells with arbor area below 50,000 μm^2 have smaller somas, less arbor length, and higher arbor density. The vertical dashed line shows the cutoff point for alpha cells (unfilled circles) and beta cells (filled circles) in our sample. (A) As arbor area increases, soma area increases. (B) Total arbor length increases with arbor area. (C) As arbor area increases, arbor length per unit area decreases.

Corresponding acuity estimates

To provide an independent confirmation of our cell density analyses, we estimated the acuity of 4-week-old chicks from calculations of the Nyquist frequencies [see Troilo et al. (1993) for details] for RGC densities across the retina in the horizontal meridian. The mean axial length of 11.05 mm (measured in this study by ultrasonography) and the posterior nodal distances across the horizontal retina as measured in chick eyes by Schaeffel and Howland (1988) were used to calculate retinal magnification. RGC density was corrected for glia and displaced ganglion cells (Ehrlich, 1981), and two equally distributed functional types of ganglion cells were assumed (ON and OFF). Based on these assumptions, and the mean RGC densities measured in this study, we calculate that the Nyquist frequency in the central retina is approximately 8.1 cycles/deg. At 40-deg temporal and nasal retinal eccentricities the Nyquist frequency is 6.6 and 5.3, respectively. These estimates are consistent with those based on cone packing (12.5 cycles/deg: Rohrer et al., 1992) and to behavioral measures of 4-6 cycles/deg (Demello et al., 1993).

Arbor structure in the normal retina

For all measured cells in normal retinas, dendritic arbor area of RGCs increases as RGC density decreases from the center to the periphery (Fig. 7). In the central retina, RGC dendrites cover a mean area of 7133 ± 9099 μm^2. In the periphery, mean dendritic arbor area is significantly enlarged to 17,006 ± 11,235 μm^2 (ANOVA, $P < 0.01$).

Ten percent of RGC sample were bistratified, with the proportion similar in central and peripheral retina. The area of the two strata covaried positively across cells ($r = 0.84$, $P < 0.0006$), although the arbor area proximal to the RGC soma consistently exceeded the area of the distal arbor (Fig. 8A).

Retinal ganglion cells in the enlarged retinas

Eye size and overall cell distribution

Visual form deprivation of one eye produces ocular enlargement and an increase in retinal area. In the 13 pairs of eyes examined, those raised with form deprivation have 14% (11.9 *vs.* 10.39 mm; paired *t*-test, $P < 0.01$) longer axial lengths and 13% wider ocular diameters (15.73 vs. 13.93 mm; paired *t*-test, $P < 0.01$) compared to the contralateral control eyes. Fig. 9 shows examples of control and expanded retinal wholemount pairs of 4-week-old chicks. Enlarged eyes had retinal areas 35% larger than controls (342 vs. 254 mm^2; paired *t*-test, $P < 0.01$). Along with the expanded retinal areas of the visually deprived eyes there is a general reduction in cell density. Fig. 6 (middle) shows examples of RGC densities from the center and mid-periphery of enlarged Nissl-stained retinas for comparison with control retinas at identical locations. As in normal retinal development, the largest reductions in cell density in an expanded retina are found in the peripheral retina. Despite the reduction in RGC density, the acuity predicted from the RGC Nyquist frequencies is slightly higher than in controls (central retina, 10 cycles/deg; 40-deg temporal 7.7 cycles/deg; 40-deg nasal 6.6 cycles/deg) because of the axial enlargement of the eye and its effect on retinal magnification.

Increase in arbor area

The dendritic arbors of the RGCs were enlarged in area proportional to the amount of retinal expansion in the visually deprived eyes (mean change in retinal area 34%; mean change in arbor area 40%; Fig. 7). The mean area of RGC dendritic arbor is significantly increased in the expanded retinas (ANOVA, $P < 0.01$) in both the center (11,009 ± 9796 vs. 7132 ± 9099 μm^2, $P < 0.05$) and the periphery (21,378 ± 10,384 vs. 16,916 ± 11,167 μm^2, $P < 0.05$) of enlarged retinas. The distributions of arbor sizes are uniformly shifted upward, with no indications of emerging bimodality (Fig. 5B). The 50,000 μm^2 cutoff for beta cells was maintained for the expanded retinas (and the experimentally depleted retinas described below) since none of the cells sampled were found to possess arbor areas between 50,000 and 70,000 μm^2 which is the predicted enlargement of a large beta cell in the experimental retinas.

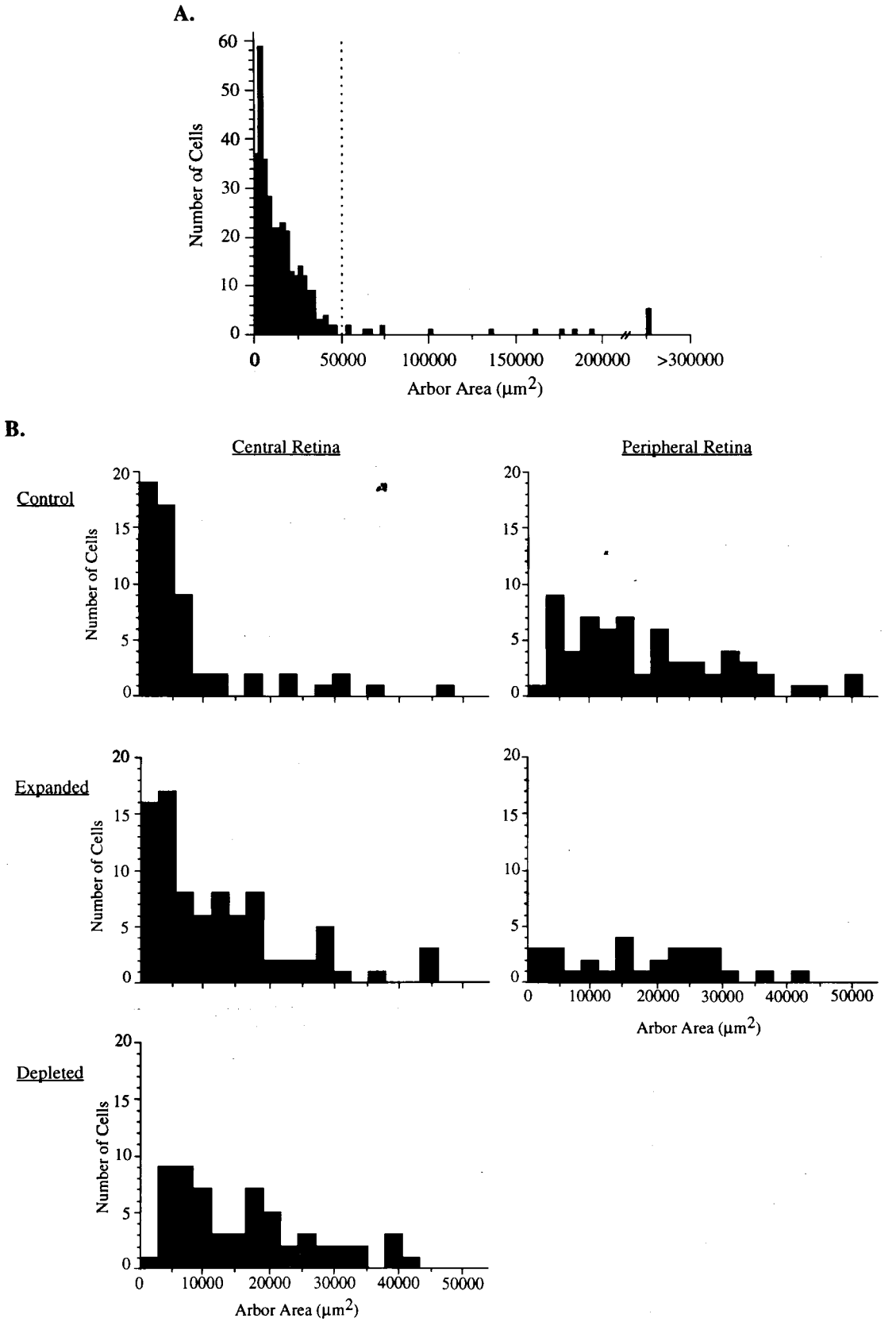

Fig. 5. The distribution of retinal ganglion cell arbor size. (A) The distribution of arbor area of all cells sampled. The dashed line represents the 50,000 μm^2 cutoff for alpha and beta cells in our sample. (B) Distributions of beta cells in control, expanded, and depleted retinas. Arbor areas from central retinas are shown in the left column and those from peripheral retina are shown in the right column. The top row shows the distributions from control retinas, the middle row shows those from expanded retinas, and the bottom row shows that from the central region of depleted retinas.

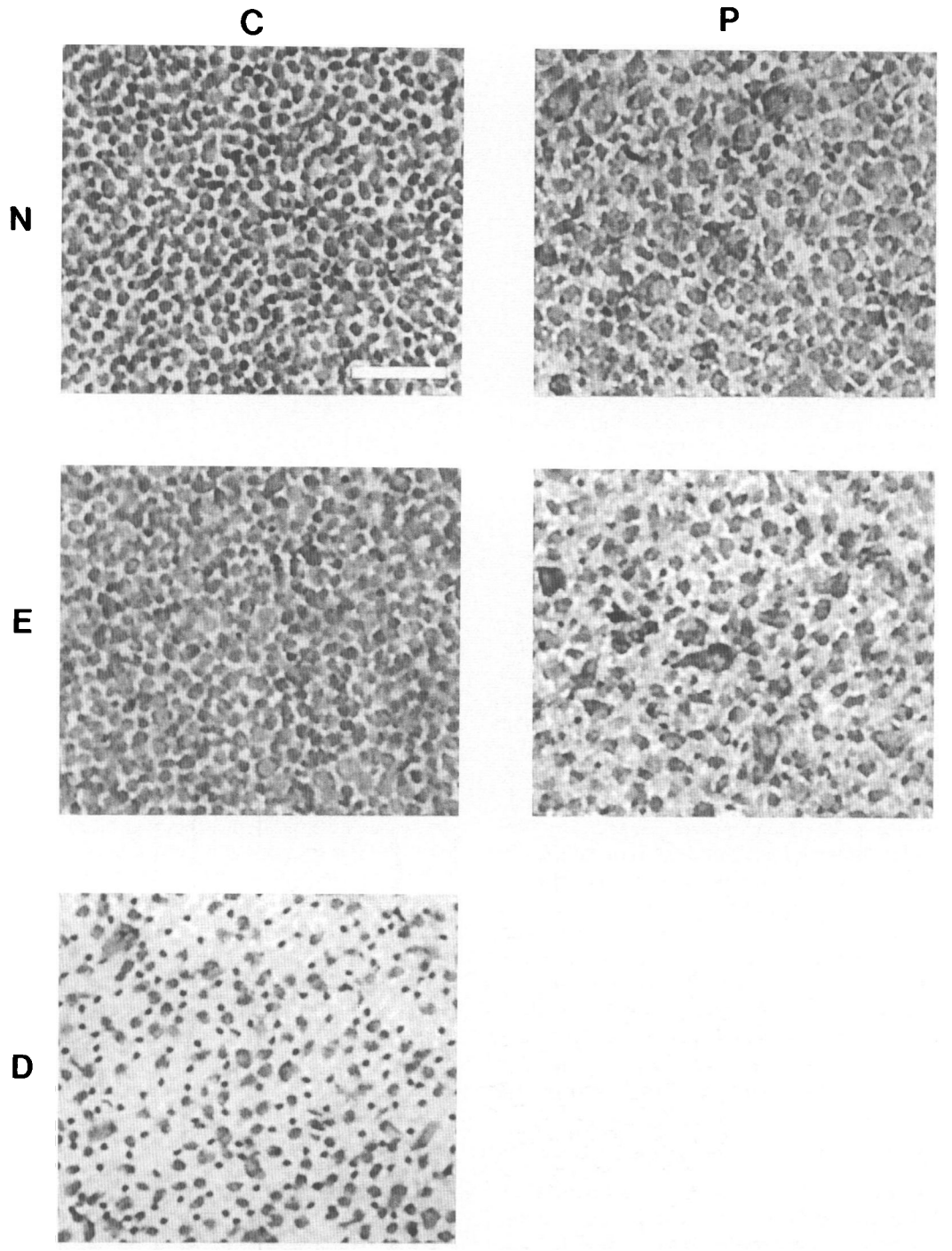

Fig. 6. Nissl-stained retinal ganglion cells in chick retinas. Left column, central retinas (C). Right column, mid-peripheral retinas (P). Top row: normal retinas (N). Middle row: expanded retinas (E). Bottom row: depleted retina (D). The RGC density is lower in the periphery of normal retinas. Compared to normal retinas, retinal ganglion cell density is decreased in expanded retinas, in both the center and periphery. Scale bar = 100 μm.

Arbor structure

In the subset of cells with arbors presenting sufficient details for additional analysis (as in Fig. 1), we explored a number of features of internal structure for evidence of altered intrinsic organization. We found none, although care should be taken not to overinterpret these results. Overall, no evidence indicated other than a simple expansion of the arbor, maintaining intrinsic geometry.

Consistent with the results in the large sample, in the smaller sample of cells from expanded retinas there was a mean arbor area expansion of 29% relative to cells from control retinas (25,707 ± 8819 *vs.* 19,941 ± 6834 μm^2, $P < 0.05$). Assuming

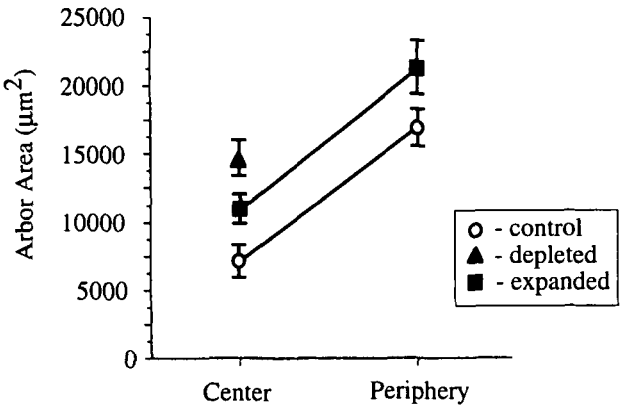

Fig. 7. Comparisons of arbor area (mean ± S.E.M.) in the central (left) and peripheral (right) regions of control (unfilled circles), expanded (filled squares), and depleted retinas (filled triangles). Note that in the depleted case, arbor area of cells in the central retina is most closely comparable to that of cells in the periphery of control retinas and in the center of expanded retinas control retinas.

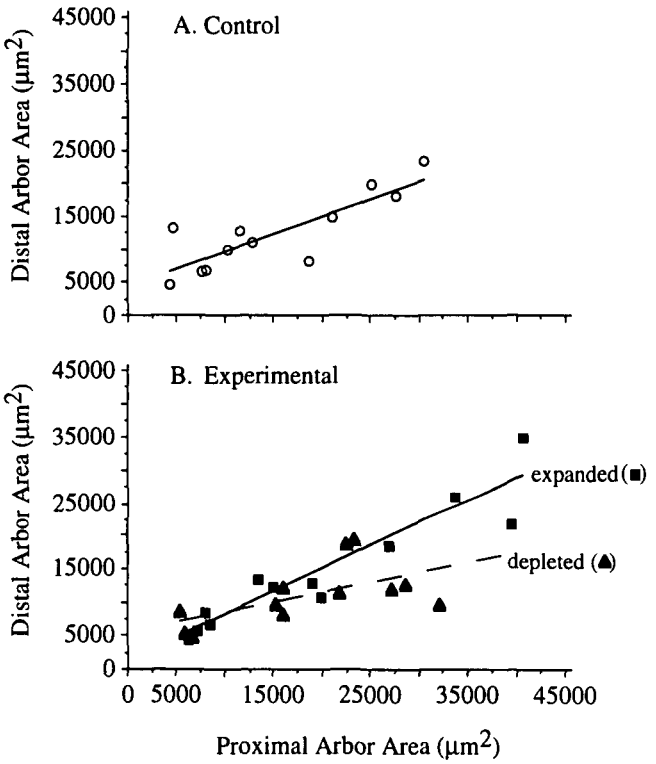

Fig. 8. The relationship between distal arbor area and proximal arbor area of bistratified retinal ganglion cells. (A) In control retinas, distal arbor area increases with proximal arbor area, but distal arbors are generally smaller. (B) As proximal arbor area increases, distal arbor area increases both in expanded (filled squares) and depleted (filled triangles) retinas. In depleted retinas, however, the difference between proximal arbor area and distal arbor area is significantly greater as shown by the lesser slope of the regression line (dashed line) compared to that from bistratified cells of the expanded retinas (solid line).

a circular arbor area, and using the geometrical ratio of a circle's diameter to area, increasing the arbor area by 29% predicts an increase in arbor length of 13.5%. Consistent with this prediction, the mean length of total arbor in the expanded group (4426 ± 1307 μm) was 14.4% larger than in the control group (3866 ± 1313 μm) though the difference between experimental and control groups was not significantly different (ANOVA, $P = 0.28$). The mean number of branches per cell did not change significantly (control = 289 branch points; expanded = 308; ANOVA, $P = 0.71$). Branches per unit area were 23% lower in the expanded retinas, though not significantly so (ANOVA, $P = 0.399$). In Fig. 11A, the relationship between branch complexity and arbor size is plotted, showing that for both normal retinas and the expanded retinas, branches per unit area decrease at the same rate with increasing arbor size. In the normal retina, the major factor contributing to arbor size is central or peripheral position in the retina. The total number of branches in a mature beta cell stayed constant in normal central and peripheral retina, and in the expanded retina, as arbor size increases.

Retinal ganglion cell morphology in the depleted retinas

Our surgical technique for partial optic nerve section results in patches of RGC depletion principally across the central retina. We selected for analysis 65 DiI-labeled cells that were in areas where the local RGC density was depleted by at least 20%. Fig. 6 (bottom) shows an example of cell density in RGC layer in a highly depleted region of central retina. In our analysis, we will make two types of contrasts (illustrated in Fig. 10). RGC depleted areas of central retina were compared to normal central retina (Fig. 10, left); in this case RGC cell density is lower in the depleted retina (63%) and available convergence from presynaptic cells is higher. In addition, we make use of the fact that our depletion procedure reduces central cell density to the approximate levels of the normal and expanded peripheral retina (Fig. 10, right). Mean RGC density in the depleted central retina (9726 cells/μm^2) is comparable to RGC density in the peripheral retina of the control (9813 cells/μm^2) and also to the enlarged (8529 cells/μm^2) cases. In this contrast, RGC density is roughly constant, but the availability of presynaptic input is much higher for the depleted central retina.

The mean arbor area of the depleted central retina is larger than the mean arbor area in the central control retina (59 cells) by 52%, (Fig. 7 control 7132 ± 9099 μm^2; depleted 14,762 ± 10,053 μm^2, ANOVA, $P < 0.01$). Comparing central depleted retina to control peripheral retina, where RGC density is comparable, RGC arbor areas are not significantly different (control periphery 16,916 ± 11,167 μm^2; depleted central 14,762 ± 10,053 μm^2, $P = 0.23$). The frequency distribution of arbor size also resembles that in the normal periphery (Fig. 5).

Arbor structure

The dendritic arbors of 23 RGCs from the depleted retina were further analyzed and compared to 14 cells from peripheral control retinas and nine from peripheral expanded retinas. Both arbor branch density and stratification pattern are altered in retinal areas of comparable RGC density but potentially increased presynaptic convergence. A simple measure of arbor complexity (branches/arbor area) is higher in the depleted group (Fig. 11; ANOVA, $P < 0.05$), and the relationship of arbor area to branch complexity still holds, but at a consistently higher level of branch density. Stratification in the depleted central retina is altered compared to the expanded retina, such that the distal arbor of the depleted retina did not increase in size in the same proportion

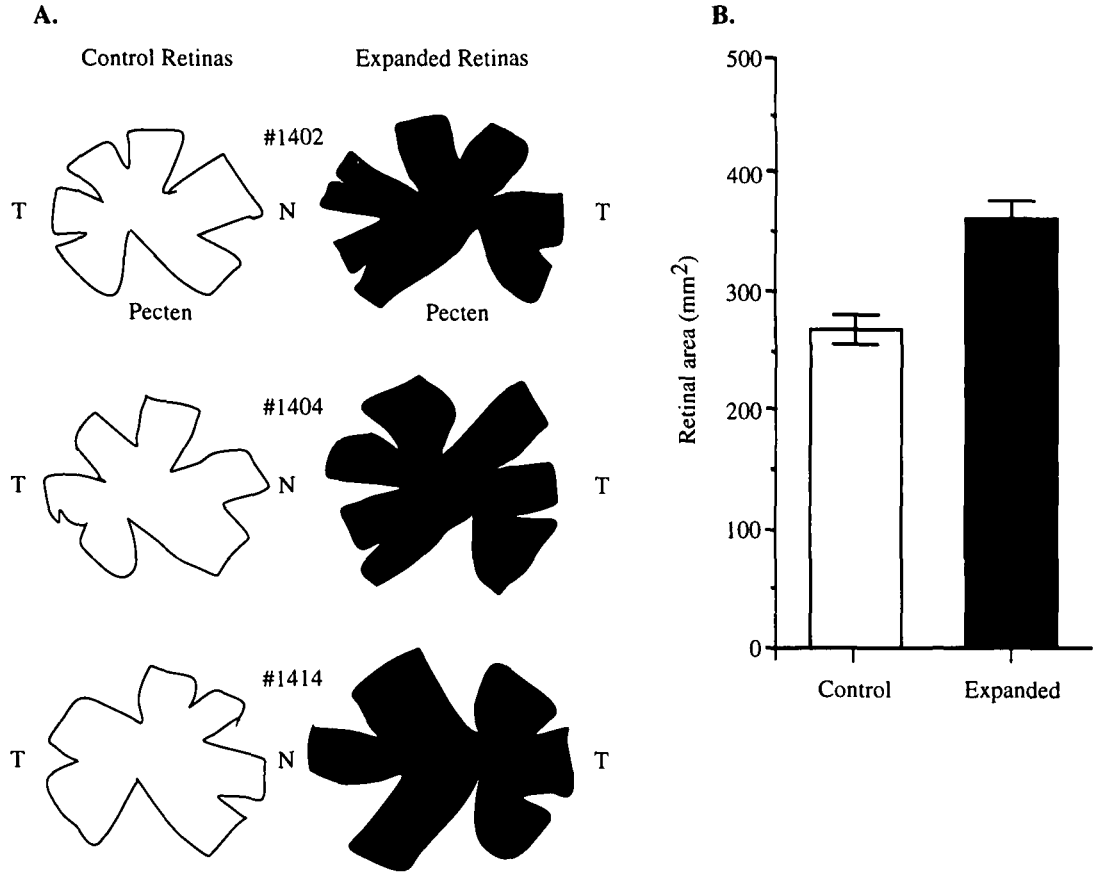

Fig. 9. (A) The effect of visual-deprivation-induced ocular enlargement on retinal area is shown in three example pairs of retinal wholemounts. Control retinas are to the left, expanded retinas to the right (T: temporal; and N: nasal). The retinal whole mounts of expanded retinas are larger than control retinas. (B) Comparison of retinal area (mean ± S.E.M.) from 13 experimental-control retinal pairs. The retinal area of eyes enlarged by visual deprivation is 35% larger than retinal area of control eyes.

to proximal arbor as in the control and expanded case; the distal arbor was always relatively smaller (Figs. 8A and 8B; control slope = 0.53; depleted slope = 0.28; expanded slope = 0.70, comparison of slopes $P < 0.05$).

Discussion

In this study, we examine the developmental control of RGC dendritic arborization in the chick. We have restricted our attention to RGCs generally categorized as beta cells on the basis of the size of their arbor areas. Retinal ganglion cells may be sorted on a number of anatomical dimensions (e.g. size, branch geometry, stratification), physiological properties, or any multivariate constellations of traits (Rodieck & Brening, 1983). Chicken RGCs have acquired at least five different anatomically based classification schemes (Ramon y Cajal, 1933; Nishimura et al., 1979; Ehrlich, 1981; Thanos et al., 1992; Snow et al., 1994). All these studies converge on a minority population of large cells with sparse, widely branched, large arbors (alpha cells); a majority population of medium-sized, highly branched cells (beta cells); and a variable number of smaller minority populations.

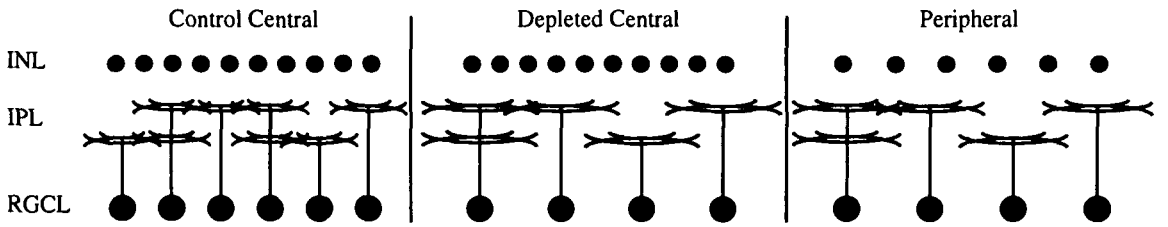

Fig. 10. Schematic drawing of convergence relationships between the RGC layer and bipolars and amacrine cells in the central region of normal retinas (left); the central region of an RGC depleted retina (center); and the peripheral region of either a normal or expanded retina (right).

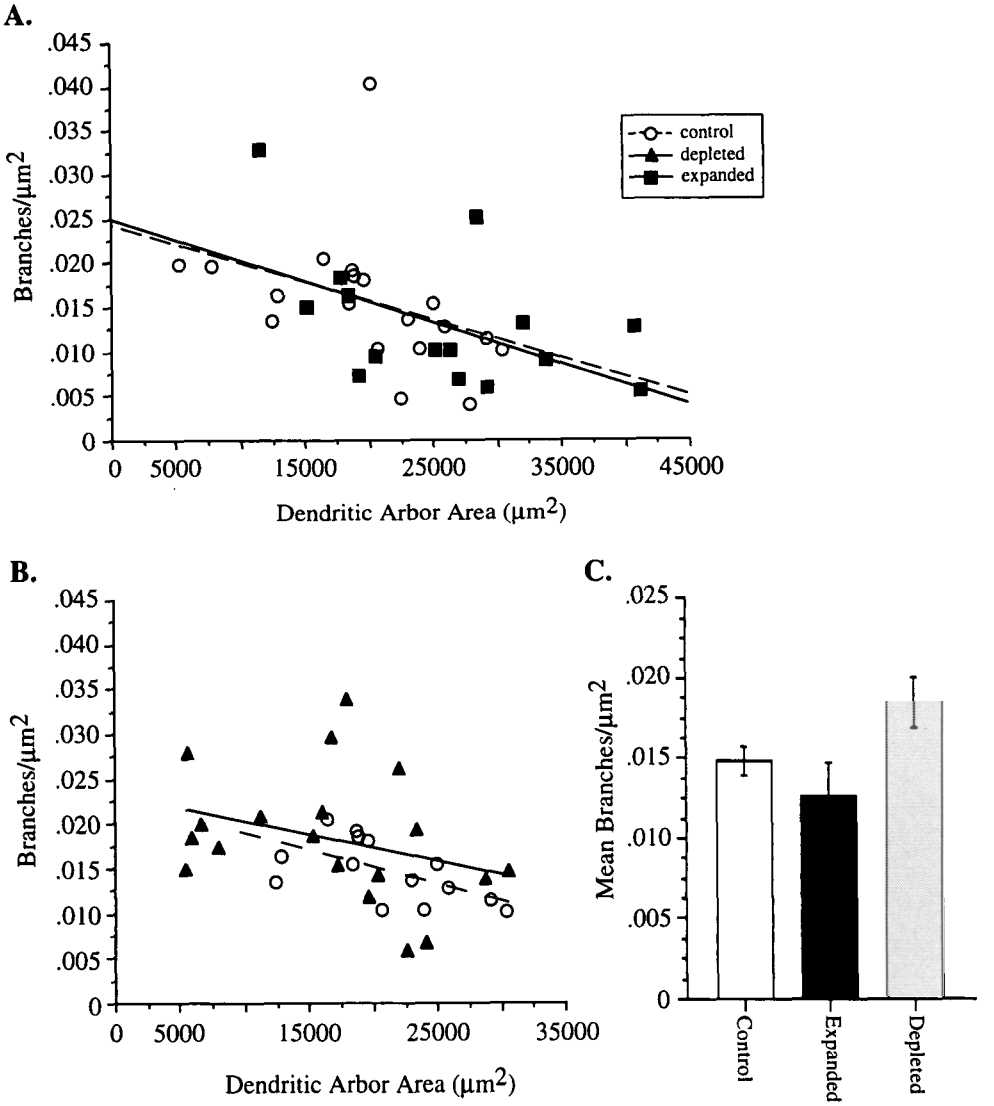

Fig. 11. The relationship between branch density (a simple measure of arbor complexity) and dendritic arbor area. (A) Branch density decreases at the same rate with increasing dendritic arbor area in control (unfilled circles, dashed line) and expanded (filled squares, solid line) retinas. (B) The relationship between branch density and arbor area in depleted retinas (filled triangles, solid line) is similar to that in control retinas (unfilled circles, dashed line), however, branch density in depleted retinas is significantly higher. (C) The bar graph shows comparisons of arbor complexity as the number of branches per area (mean ± S.E.M.) among control, expanded, and depleted retinas.

Retinal ganglion cell density and availability of presynaptic input influence different aspects of beta RGC growth in the chick. When beta RGC density is experimentally decreased, whether by retinal expansion or by loss of RGCs, their arbors increase in size corresponding to the local RGC density. If, however, the density reduction is paired with an increase in potentially available bipolar and amacrine cell input, arbor structure is altered, resulting in a greater number of branches per arbor area and a change in the relative sizes of inner plexiform layer (IPL) stratifications in bistratified cells.

We believe that the changes observed are related to the normal mechanisms controlling the maturation of arbors and the formation of synapses in the postnatal developing retina. Though the chick is precocial at hatching, and able to use vision to peck accurately at seeds and for imprinting, ocular size is approximately 80% of that in adults and retinal growth is still underway. Although the relationship of chick RGC arbor structure at hatching to structure at maturity remains to be fully studied, we do know that major developmental milestones in the chick visual system occur at the same relative times as corresponding events in several well-studied mammals (Dreher & Robinson, 1988). We can infer from this work that the posthatch period in the chick should be a period principally of dendritic elaboration and growth; cell death in the ganglion cell population is a prehatch event (Rager & Rager, 1976) and the major regressive events of spine retraction (Ramoa et al., 1988) and initial process retraction (Bodnarenko & Chalupa, 1993) should be mostly concluded.

What happens to the cells, arbors, and synapses of amacrine and bipolar cells in the case of overall reduced cell density or specifically reduced ganglion cell number is largely unknown. Prior studies (Beazley et al., 1987; and see Finlay, 1992 for

review), and our own work in progress (Xiong et al., 1995), suggest that neither the number of amacrines (both generally and specifically identified) nor bipolars change as a result of these manipulations. The arbor structure in the inner plexiform layer of both bipolars (Crowley et al., 1995) and tyrosine-hydroxylase immunoreactive amacrine cells (Teakle et al., 1993; Xiong et al., 1995) change in these conditions, suggesting that every element in the retina responds dynamically to its convergence environment.

An interesting mechanistic question about deprivation myopia is the role of deprivation *per se*, rather than the stretch induced by retinal enlargement, in the alteration of retinal ganglion cell structure. If vision-dependent regressive events are in progress, several outcomes are possible depending upon precise timing. For example, as in the development of the mammalian visual cortex during early visual experience, visual deprivation might delay the critical period for dendritic remodeling and sculpting and produce retinal ganglion cells with larger dendritic trees. With deprivation later in development, cells will not undergo as much dendritic growth and have smaller arbors (Greenough, 1986). There are several ways of blocking deprivation-induced myopia (e.g. Stone et al., 1989; Rohrer et al., 1993). These could be used to produce an eye deprived of form vision for a comparable period, but of normal size. If the effect of form deprivation is to retard the growth of dendritic arbor, our failure to find increased branching in the expansion case could be attributed to reduced activity, not unresponsiveness of branching to increased retinal area. The similarity of branching in the expanded retina to branching in the peripheral retina of a normal eye, however, makes this possibility unlikely.

The intent of this study was to determine if the two developmental manipulations of RGC density have substantial effects on arbor size and some gross features of arbor structure. Finding fully isolatable cells using DiI, as is also true of most intracellular injection techniques, appears to bias the sample to a population of somewhat larger cells than the population mean. The reasons for this are not fully understood. Nevertheless, since ratio relationships between treatment groups are maintained between our full cell population and the isolatable cells (Fig. 5), and since features of arbor structure scale quite regularly with arbor size, we can be reasonably certain that the features of arbor structure we measured are characteristic of the entire population. In future studies, we will use intracellular filling to target particular size ranges of retinal ganglion cells and further explore arbor structure at the light- and electron-microscopic level.

The nature of process growth

At least two, and possibly three, separate kinds of cell growth are suggested by the studies reported here: (1) interstitial membrane extension resulting in larger cells but conserving geometry; (2) distal process extension by growth cones to cover space produced by reduced ganglion cell competition; and (3) branching by growth cones produced by availability of presynaptic terminals (Maslim et al., 1986). As axons develop, process extension and branching can be distinguished as two separate kinds of growth but, in the retina, distal process extension and branching of dendrites could presumably be manifestations of the same mechanism, and inseparable. Increases in cell size by stretch of the cell, resulting in incorporation of new membrane without growth cones, is necessary in most neural systems as the brain and peripheral nervous system grow. In the retina, simple expansion of a cell, preserving intrinsic geometry, has been described for the expansion of retinal ganglion cells of the goldfish eye (Bloomfield & Hitchcock, 1991; Hitchcock, 1993) and the several cell classes of the rabbit retina (Deich et al., 1994). For rabbit and cat retinal ganglion cells, the type of change in arbor structure occurring with retinal growth appears to be a hybrid of interstitial membrane extension and terminal, growth-cone-mediated growth (Dann et al., 1988; Wong, 1990; Deich et al., 1994). In the present case of deprivation-induced retinal stretch, our results are consistent with simple interstitial membrane extension, although a finer analysis of the geometry of terminal branchlets would be required to rule out a component of terminal growth. Though interstitial growth retains arbor geometry, it will not retain dendritic cable properties unless synaptic junctions are actively produced and rearranged (Bloomfield & Hitchcock, 1991). It is interesting to consider what the signal for the generation of new postsynaptic specializations might be, and to determine if addition of new synapses is adequate to maintain cable properties, or whether the gain of individual synapses must be altered as well.

We see two types of active growth to account for in this study: increased arbor area when retinal ganglion cell competition is reduced, and increased internal branching when a greater number of amacrine and bipolar cells are available for input to a RGC. A common mechanism proposed for induction of branching is a trophic response to factors produced by empty presynaptic sites. A similar mechanism could also be considered for the interstitial growth and growth-cone-mediated growth increasing arbor circumference. If retinal expansion mechanically decoupled some presynaptic and postsynaptic sites, the resulting decreased activation of the postsynaptic cell could signal dendritic growth. The signal for branching resulting in increased arbor complexity when more synaptic sites are available would not necessarily depend on reduction of the activity of the postsynaptic cell.

We do not know what the change in response properties of cells in depleted retinas might be. Retinal ganglion cells of equivalent arbor size have more internal branching when more potential amacrine and bipolar input is available. Specificity of response properties could be reduced, or responsivity could be enhanced. Alternatively, if activity-dependent sorting strictly gates what input a retinal ganglion cell may accept, receptive-field properties might stay stable, as they do in the superior colliculus when the retinal projection is "compressed" (Pallas & Finlay, 1989). In this case, the addition of synapses could be adjusted downward to preserve normal responsivity.

One interesting constraint on growth that might mold features of retinal circuitry is an intrinsic limit to growth or size of retinal ganglion cell arbors. Within the range of the perturbations of retinal growth studied here, we have found no evidence for such a limit on retinal ganglion cell arbor, with one possible exception. In all cases, retinal ganglion cell arbors expand to fill available space. Most telling is that bistratified arbors have the same mean diameter as monostratified arbors, and that the size of the two arbors strongly covary; larger proximal arbors are associated with larger distal arbors in any one cell. The one possible exception is the reduced size of the distal arbor in retinal ganglion cells of depleted retinas which have higher increased internal branch number; this could indicate a ceiling in the branching capacity of an individual cell. Similarly, recent work shows a strong conservation of arbor in bipolar cells which may provide a strong constraint on retinal organization (Crowley et al., 1995).

Differences in the regulation of convergence in the superior colliculus and visual cortex

Regulation of convergence between afferent and postsynaptic populations to construct receptive-field properties has been studied fairly extensively in connections from RGCs to superior colliculus and lateral geniculate to primary visual cortex. Even at this initial level of analysis, the retina offers several interesting contrasts. If the input to the colliculus is made abnormally dense, or abnormally sparse, it is the presynaptic element (the retinal ganglion cell axon) that regulates its arbor size to conform to the altered condition; synaptic density, cell size and arbor (except in cases of severe denervation), and receptive-field properties of the postsynaptic cell remain constant (Hayes & Meyer, 1988; Xiong & Finlay, 1993; Xiong et al., 1994). In the visual cortex, establishment of ocular dominance columns is accomplished for the most part by the sorting of axons, not the reconformation of cortical cell dendritic arbors. Similarly, orientation selectivity is not produced by the extension of dendritic arbors along the appropriate topographic axis, but by sorting a small number of appropriately topographically aligned lateral geniculate afferents onto a visual cortical neuron (Stryker, 1991). Although in both the visual cortex and superior colliculus dendritic architecture may be made to change principally through manipulations that change the gross activity of the network, in the case of convergence cortical and collicular neurons act as if they are fixed in their general morphology.

How common the developmental mechanisms observed in RGC dendrites are to the rest of the nervous system is unclear. The functional requirements of the sensory retina (to adequately sample the visual field at appropriate sensitivity), and its location within the growing eye, may necessitate special developmental mechanisms. We have shown that, in the development of RGC dendritic arbor, growth is responsive to "available space" and the availability of potential afferent inputs. Generation of postsynaptic processes in response to an increased availability of presynaptic elements in the retina may be fundamentally different from what occurs in other parts of the visual system (e.g. cortex and colliculus), and might require a different arrangement of mechanisms for activity-dependent stabilization and growth. In the retina, the postnatal growth of the matrix, as represented by the enlarging surface area, is related principally to the enlarging eye and not generated directly by the growth of retinal neurons. By comparison, in the developing colliculus and cortex, the growth of the matrix and "available space" is generated mainly by the proliferation of cells and their processes. Later in development, however, enlargement of the colliculus and cortex occurs after the generation of neurons has ceased. Furthermore, dendritic invasion of cell-depleted zones and avoidance of inactive regions has been demonstrated in several systems (Crandall et al., 1990; Goldstein et al., 1993; Kossel et al., 1995). So the growth of RGC dendrites seen in this study may represent a generic cell process in an unusual environment.

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