Differential Dendritic Shrinkage of α and β Retinal Ganglion Cells in Cats with Chronic Glaucoma

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Purpose. To study changes in the dendritic morphology of retinal ganglion cells (RGCs) in cats with experimental chronic glaucoma.

Methods. Chronic elevation of intraocular pressure (IOP) was produced by injecting endogenous ghost red blood cells into the unilateral anterior chamber of the feline eyes for 1 month. The morphologic features of retrograde-labeled RGCs by bilateral injection of horseradish peroxidase (HRP) into layers A and Aa1 of the lateral geniculate nucleus (LGN) were examined and compared between the normal and glaucomatous eyes. Nissl staining was used for measuring the change in cell density in the retina and the LGN.

Results. Quantitative analysis of 720 labeled α and β type RGCs showed that the cell density, body size, maximum dendritic field radius, total dendritic length, and number of branch bifurcations of dendrites decreased significantly in glaucomatous eyes compared with normal ones. The cell loss and shrinkage of dendrites in α type ganglion cells in the retina was more pronounced than that in β type cells. The cell density of all kinds of cells in the retina and LGN monotonically declined with time while IOP was elevated, and cell loss was more significant in large cells than in small ones.

Conclusion. Progressive cell loss and dendritic damage by chronic elevation of IOP in RGCs and LGN cells are more pronounced in the Y-channel (large cells) than the X-channel (small cells) in feline glaucomatous eyes. The dendritic structure changes and corresponding physiological deficits of RGCs occur before cell death and thus may provide an opportunity for clinical treatment. (*Invest Ophthalmol Vis Sci.* 2003;44:3005–3010) DOI:10.1167/iovs.02-0620

Glaucoma is the most common cause of blindness except for cataracts. In most cases, glaucoma is characterized by an elevation of intraocular pressure (IOP), progressive changes in morphology of the optic disc and retinal axon layer, and visual field defects. Eventually, it makes the eye blind by killing retinal ganglion cells (RGCs). Many studies have shown that glaucoma and chronic elevation of IOP cause degeneration in the optic nerve fibers[1–4] and progressive loss of retinal ganglion cells.[4–6] Weber et al.[7] have reported that in glaucomatous monkeys, the mean soma sizes of both midget and parasol retinal ganglion cells decreased significantly, but only parasol cells showed a significant reduction in dendritic field size and axon diameter. Because dendritic structure decides spatial and temporal property of a retinal ganglion cell in visual information processing[8–9] and the changes in dendritic morphology in the glaucomatous eyes come before cell death,[7] it is important to study dendritic change in greater detail.

In the cat's retina, α and β ganglion cells are classically defined as distinct morphologic types that correspond to physiological Y- and X-type cells, respectively. Y (or α) cells have the largest soma, the largest dendritic field area, and the thickest dendrites and preferentially respond sensitively to stimuli of low contrast, low spatial frequency, and fast-moving patterns. In contrast, X (or β) cells have a medium soma, the smallest dendritic field area, and a bushy dendritic arbor and are sensitive to stimuli of higher contrast, fine structure, and relatively low velocity of motion.[10–14] Both Y and X RGCs project separately to Y- and X-type relay cells in layers A and A1 of the dorsal lateral geniculate nucleus (LGN) and form the parallel pathway of visual information processing in the cat,[12–14] similar to the magno- and parvocellular pathways in the monkey's visual system.

It is well-documented in monkeys with chronic glaucoma that large RGCs are more seriously damaged than small cells.[2–4] However, previous studies in our laboratory have demonstrated that in the cat, the Y-type ganglion cells in the retina and relay cells in the LGN are more tolerant than X cells to brief elevations of IOP.[15–17] These findings in acute IOP elevation are in conflict with many observations in eyes with chronic glaucoma. Whether this opposite observation is caused by differences between species or by the different period and strength of the IOP elevation remains unknown. In the current study, we examined the detailed changes in dendritic structure and cell density between the α and β ganglion cells in cats with chronic glaucoma. The results indicate that, as in primate RGCs, α cells (large cells) in the cat's retina are more impaired than β cells (small cells), in the chronically glaucomatous eye.

Methods

Animal Model of Chronic Glaucoma

Fourteen adult cats weighing between 2 kg and 2.7 kg were used as experimental models of glaucoma. Their eyes were tested to ensure ophthalmic health. All the procedures for production of chronic elevated IOP were conducted according to the description by Quigley and Addicks.[18] The glutaraldehyde-fixed autogenous red cells in saline (1:1 in volume) of 0.3 mL was injected into the anterior chamber of the unilateral eye of a cat when another syringe was used to drain off the same volume of aqueous humor. The contralateral eye of each cat was used as the control. The IOP of each eye was repeatedly measured using a Schiotz tonometer in cats under ketamine anesthesia. The

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animals recovered from anesthesia, and IOP was routinely measured daily for the first 3 days and then once every 2 to 3 days for 1 month. All investigations involving animals conformed to the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the policy of the Society for Neuroscience on the Use of Animals in Neuroscience Research.

Animal Preparation

Six cats with experimental glaucoma were prepared for horseradish peroxide (HRP) retrograde labeling of RGCs and another eight cats for Nissl staining of RGCs and LGN cells. Animal preparation has been described in detail in previous studies.[15–17] They were sedated with intramuscular injection of ketamine (25 mg/kg). Intravenous and tracheal cannulas were inserted, and all pressure points and incisures were infiltrated with a long-acting anesthetic (1% lidocaine HCl). Animals were fixed on a stereotaxic apparatus. A mixture of gallamine triethiodide (8–10mg/kg per hour) and urethane (15–20 mg/kg per hour) was continuously infused intravenously to maintain anesthesia and paralysis. Artificial respiration was maintained to keep the animals alive. The end-tidal CO_2 was measured and kept at approximately 4% by adjusting the rate and volume of the respirator. Electroencephalograms and electrocardiograms were monitored to evaluate the depth of anesthesia. The body temperature of the animal was monitored and kept in normal physiological condition throughout the experiments. The pupils were dilated with atropine (0.5%) and the nictitating membranes were retracted with phenylephrine HCl (2%). The eyes were refracted carefully and corrected with appropriate contact lenses; artificial pupils (3 mm in diameter) were also used.

The single-unit electrophysiological recording and visual grating stimulation were identical with those described in detail in the previous publication.[15,16] The optic nerve head, vessels, and area centralis of the retina were mapped on a white screen 114 cm from the cat's eye, using the method of fundus reflective projection.[19] The visual receptive fields of the relay cells in the LGN were plotted onto the white screen with a handheld target. Thus, the layers of the LGN and stereotaxic location of the microelectrode tip could be identified according to the classic work of visual field projection to the cat's LGN by Sanderson.[20] Soon after withdrawal of the microelectrode from the LGN, 2 μL HRP (in PBS and 1% dimethyl sulfoxide [DMSO]; pH 7.4) each was slowly injected into five to six places within layers A and A1 of the LGN through a 10-μL microsyringe fixed on the stereotaxic apparatus.

HRP Histochemistry Reaction

Animals survived for 48 hours after the HRP injection. Under deep anesthesia, animals were infused through the heart, first with 1000 mL saline containing 1% heparin, and then with 2000 mL saline containing l% paraformaldehyde, 2.5% glutaraldehyde, and 0.1 M PBS [pH 7.4], and finally with 1200 mL saline containing 10% sucrose and 0.1 PBS (pH 0.4). The brain was removed. The portions containing the LGN were blocked and stored in a 30% sucrose solution for 3 to 4 days before frozen sectioning. The 80-μm sections were collected and stained with 0.05% thionine at pH 4.0 and coverslipped.

The whole retina was removed and processed immediately after the infusion. The conventional tetramethylbenzidine-sodium tungstate (TMB-ST) method was used for demonstrating the RGCs' morphology.[21]

Morphologic Analysis of RGC Dendrites

Because layers A and A1 receive projections only from β (or X) and α (or Y) ganglion cells in the feline retina, the HRP retrograde-labeled cells we observed must have been of the β and α types. Their entire morphology, including fine dendritic structure was observed by adjusting microscope focus and was drawn on a piece of paper by camera lucida. At each sampling point in a retina, at least four cells of each type of RGC were randomly chosen for measuring dendritic structure. For each eccentricity in a retina, data of four sampling points in different meridians were measured and averaged. The cell density, cell size, maximum dendritic diameter, total dendritic length, and number of dendritic bifurcations were quantitatively measured and analyzed on computer for the IOP-elevated and normal eyes.

TABLE 1. IOP Measured during Chronic Elevation

Cat	Duration (d)	Mean IOP (mm Hg)	Maximum IOP (mm Hg)
1	14	39.8	53.6
2	16	31.7	37.2
3	19	24.8	30.4
4	22	27.9	37.2
5	23	32.5	43.4
6	25	32.9	46.9
7	6	24.5	28.0
8	7	27.7	43.4
9	11	39.5	58.0
10	14	34.5	46.5
11	16	29.9	37.2
12	19	29.6	37.2
13	24	36.2	53.6
14	28	31.6	46.5

Cats 1 to 6 were used for HRP labeling of retinal ganglion cells. Cats 7 to 14 were used for Nissl staining of RGCs in the retina and neurons in the LGN.

Nissl Staining of Retina and LGN

Conventional procedures were performed for Nissl staining of the wholemounted retina and the coronal sectioned LGN slices to analyze cell density at different positions. RGC density was measured and averaged from four to six points of equal eccentricity, around which cells were counted within a 100-μm^2 area in the retina. The LGN cell density of each cat was measured and averaged from layer A1 only, in 15 to 20 coronal sections with 320-μm intervals between.

Cell Classification

According to the criteria of Boycott and Wässle[10] and Fukuda and Stone[11] such as soma size, dendritic field, and branches, the HRP-labeled RGCs projecting to layers A and A1 were easily classified into β and α types. They correspond to physiological X and Y type RGCs, and send their outputs to X- and Y-type relay cells of the LGN, respectively.[12–14] In layers A and A1 of the cat LGN, there are only two morphologic types of cells.[22] The large Gulliery type I cells are believed to correspond to Y-type relay cells, and the small Gulliery type II cells are thought to correspond to X-type relay cells in layer A1. They were easily differentiated under a light microscope.[22,23]

RESULTS

Cell Density in Cats with Glaucoma

The IOP was measured easily in each cat by Schiotz tonometry, as shown in Table 1. The IOPs measured in 1 month ranged from 24.5 to 39.8 mm Hg, (mean, 31.7 $\pm$ 4.3 mm Hg) in the 14 eyes with experimental glaucoma. Mean IOP in 22 normal eyes was 18.8 $\pm$ 1.5 mm Hg.

Generally, the measured size of the glaucomatous eyes was larger (approximately 5%–10% larger in diameter) than that of normal eyes, because of chronic elevation of IOP. Both β and α cells decreased clearly in cell density and dendritic structure everywhere in the glaucomatous retina (Figs. 1, 2). The mean and peak cell densities decreased significantly to 47.5% $\pm$ 5.6% (mean $\pm$ SD) and 47.7% $\pm$ 4.7% of the normal for α cells respectively, and 72.0% $\pm$ 6.3% and 64.6% $\pm$ 5.5% for β cells (Figs. 3A, 3D). The decline of cell density in α cells was relatively uniform everywhere in the retina and more significant than that of β cells (t-test, all $P \leq 0.05$) indicating a more

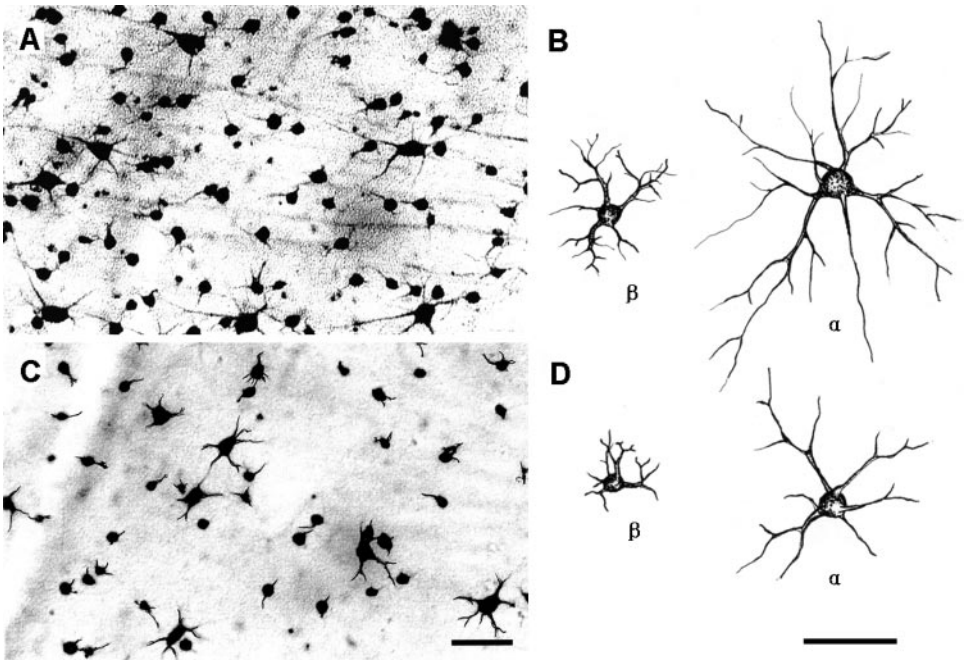

FIGURE 1. Micrographs (**A**, **C**) and the camera lucida drawings (**B**, **D**) showing the α and β RGCs labeled by injecting HRP into layers A and A1 in the dorsal LGN in a cat with elevated IOP elevation (mean IOP = 32.9 mm Hg) in one eye for 25 days. (**A**, **B**) α (the largest size) and β (the small size) RGCs in the normal eye. (**C**, **D**) α and β RGCs in the glaucomatous eye. Micrographs were taken in the temporal retina at an eccentricity of 4 mm and the drawings were made at 8 mm. Note that the cell density, cell size, and dendritic tree of both types of cells in the normal retina (**A**, **B**) were significantly greater than those of the glaucomatous retinas (**C**, **D**). Scale bars, 100 μm.

significant effect of elevated IOP on α-cell death than on β-cell death.

Notably, the rate of cell death in the glaucomatous eyes varied in different meridians in the retina. There was a tendency for the greatest cell loss to occur at the nasal meridian ($-43.5\% \pm 4.1\%$ of control) and the least loss at the superior meridian ($-34.6\% \pm 3.6\%$), although the loss was not significant (*t*-test; $P = 0.065$). This finding is in agreement with the clinical symptom of visual field defect in many patients with chronic glaucoma.

Progressive Cell Death in the Retina and LGN

Cell densities of β- and α-type RGCs and the LGN cells both decreased monotonically with duration of IOP elevation (Figs. 3B, 3E), indicating progressive cell death. Overall, the effect of elevated IOP on mean cell death in the LGN was less significant than that in the retina (*t*-test, $P = 0.043$). In the retina, the decline of relative density of α cells was statistically more significant than that of β cells throughout the period of 1 month under elevated IOP (*t*-test, all $P \leq 0.033$). At the end of the period, the cell densities of β- and α-type RGCs reduced to $51\% \pm 4.2\%$ and $33\% \pm 5.8\%$ of normal, respectively, showing a statistical difference from the normal (*t*-test, both $P \leq 0.005$). For comparison, the time course of cell death in layer A1 of the LGN was measured (Fig. 3E). After the first week of IOP elevation, the large Guillery class I cells, which correspond to the physiological type of Y cells in cats showed lower cell densities than the class II cells (*t*-test, all $P \leq 0.05$), suggesting a second-order effect of retinal cell death on the LGN cells. At the end of 1 month of elevated IOP, the lowest cell densities of class I (65% of normal) and class II (76% of normal) cells differed significantly in the glaucomatous eyes (*t*-test, $P = 0.046$).

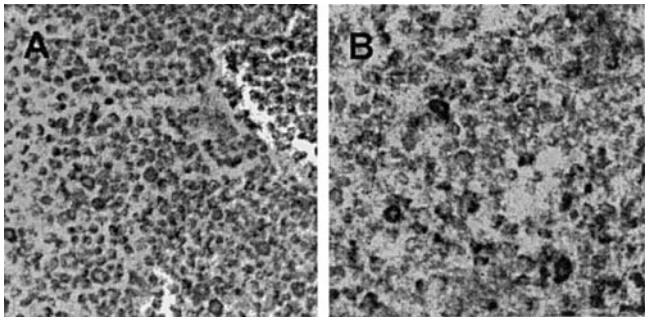

FIGURE 2. Photographs showing the densities of the α and β RGCs revealed by Nissl staining in the normal (**A**) and glaucomatous (**B**) eyes in a cat with one eye having a mean IOP of 36.2 mm Hg for 24 days. The cell density was higher in the normal (**A**) than in the glaucomatous (**B**) retina. Scale bars, 100 μm.

Change in Soma Size

The mean soma size (sectioned area) of both β and α cells in the glaucomatous eyes decreased dramatically at any position of the retina, compared with the normal eyes (*t*-test, all $P \leq 0.032$, Figs. 3C, 4F) despite the natural increase of soma size with retinal eccentricity. Notably, the mean cell body shrinkage ratio in α cells (-45%) was very close to that of β cells (-43%; *t*-test, $P = 0.09$). In contrast, significant changes in dendritic structure between the two types of RGCs were found in the glaucomatous eyes, as shown in Figure 4.

Shrinkage of Dendritic Structure

Similar to the change in soma size, the maximum radius of RGC dendritic fields increased with the retinal eccentricity, both in the normal and IOP-elevated eyes. However, all the maximum radiuses of the β and α cells in glaucomatous eyes were significantly shorter than the counterparts of the normal eyes at any position in the retina (*t*-test, all $P \leq 0.047$; Figs. 4A, 4D). The mean atrophy ratio of the maximum dendrites of α cells (-41.6%) was more significant than that of β cells (-34%; *t*-test, $P = 0.017$).

The total lengths of RGC dendrites of both β and α cells in the glaucomatous eyes shortened significantly compared with those in the normal eyes (Figs. 4B, 4E; *t*-test, all $P \leq 0.05$). Although the total length increased with eccentricity, dendritic atrophy was exhibited more remarkably in the glaucomatous eye. Again, the mean of total dendritic shortening in α cells

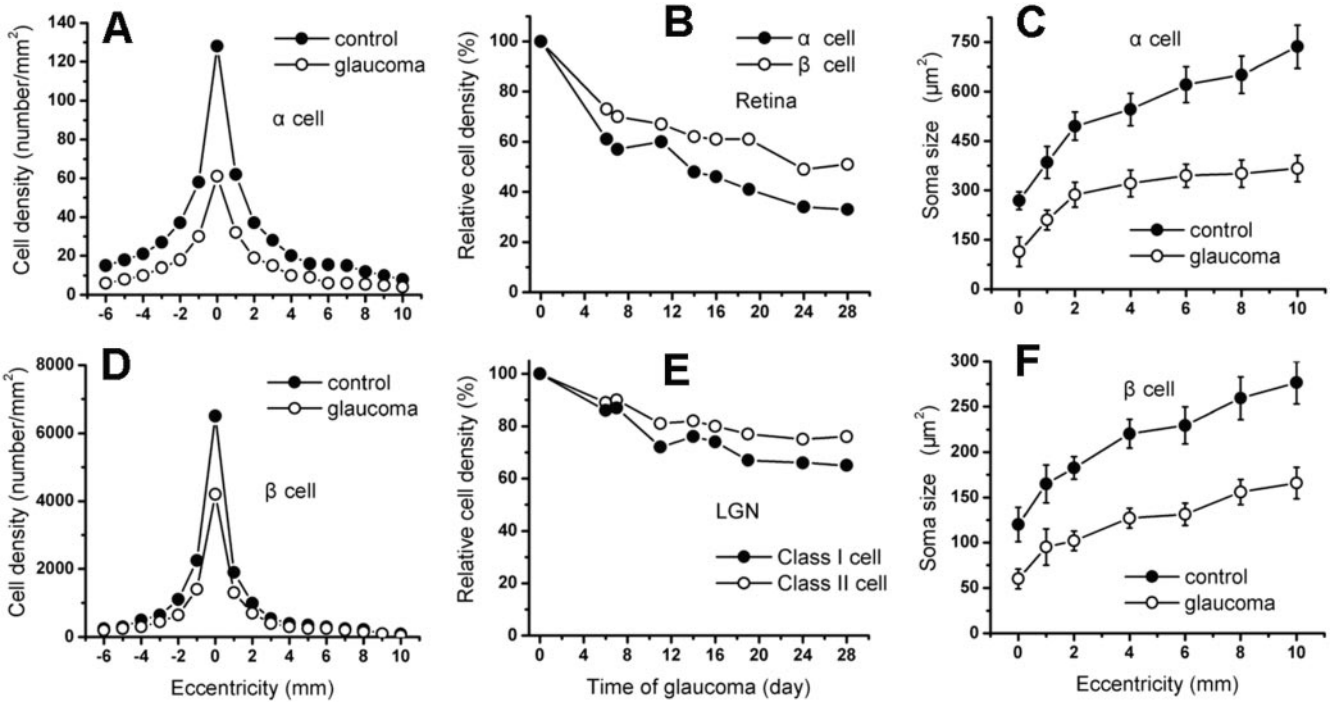

FIGURE 3. Spatial distributions of cell density (**A**, **D**) and soma size (**C**, **F**) and time course of cell death in the different types of RGCs (**B**) and LGN neurons (**E**) in normal and glaucomatous eyes. (**A**, **D**) Distribution of cell density of RGCs. Data are from one eye of three cats with unilateral elevation of IOP (mean IOP = 36.1 mm Hg) for approximately 2 weeks. Positive and negative eccentricities represent the distance from the cat's area centralis in the nasal and temporal retina, respectively. For clarity, error bars showing SDs are not shown. All were less than ±7% of the mean. (**B**, **E**) Time courses of cell density of RGCs of the glaucomatous eye and the ipsilateral LGN cells in layer A1. Data were taken at an eccentricity of approximately 2 mm in the retina and from layer A1 of the LGN in eight cats with elevated IOP (mean IOP = 31.7 mm Hg) for different durations. In the LGN, all stained cells showed less cell death than RGCs. (**C**, **F**) Distribution of soma size of RGCs. Data averaged from six cats with 14 to 25 days of elevated IOP (mean IOP = 31.6 mm Hg). Error bars, SD. Note that the relative declines in density of α cells of the glaucomatous eyes were more significant than that of β cells, but there was no significant difference in relative decline in soma size between the two types of cells.

(−63.4%) was more significant than that of β cells (−58.6%; *t*-test, $P = 0.046$).

Finally, analysis of dendritic complexity showed that in the glaucomatous eyes, the number of dendritic bifurcations of both types of RGCs decreased a great deal everywhere in the retina (*t*-test, all $P \leq 0.05$), though they increased significantly with retinal eccentricity in all glaucomatous eyes (Figs. 4C, 4F). This simplification of dendritic structure was more significant in the peripheral retina. As usual, this simplification of dendritic complexity was more significant in α cells (−59.5%) than in β cells (−44.2%) on average (*t*-test, $P = 0.018$).

DISCUSSION

In the cat's retina, approximately 90% of ganglion cells have their oriented dendritic fields which form the structural basis of orientation sensitivity of RGCs.[8,24,25] This may provide an intrinsic framework through the retinogeniculocortical pathway for the significant orientation selectivity and organized orientation columns in the visual cortex, by which the brain establishes the neural basis of form perception.[26–29] In the study, we found significant shrinkage in dendritic structure and cell body of remaining α cells and β ganglion cells in the glaucomatous eyes in addition to the clear death of RGCs. Dendrites and cell body of a neuron are the main sites receiving inputs, either excitatory or inhibitory, from presynaptic neurons. Their great shrinkage in the RGCs during early stage of chronic IOP elevation must affect the visual function from retina to brain.

Of interest, α cells showed the effects of chronic elevation of IOP more than did β cells, in decline of cell density, dendritic length, and complexity throughout the retinas of the glaucomatous eyes. This means that compared with the small β cells, which concentrate more in the central retina, the large αRGCs die more and receive relatively fewer inputs or signals from presynaptic neurons through the surface of their somas and dendrites. Because the α (Y) and β (X) cells in cats correspond to A and B cells in monkeys and large and small cells in humans,[2–4,6,30,31] The findings in cats are comparable to reports in monkeys that the large ganglion cells are selectively lost and their axonal transport from RGCs to the LGN is blocked in chronic experimental glaucoma. Furthermore, this in agreement with the symptoms usually found in patients with chronic glaucoma, who gradually lose their vision in the peripheral visual fields, and finally are left with little central vision. Hence, we conclude that it is not the difference in the experimental animal species used, but the differences in the duration and strength of IOP elevation that cause the differential effects on the α and the β (or large and small) cells. The cat model used herein allows further research in a much more convenient animal. Measurement of IOP Schiotz tonometry is available for humans, but was useful in showing relative IOPs in cats in the experiments, although the method did not provide the actual absolute values for cats because of the difference in ocular structural properties, such as scleral rigidity, between cats and humans.

In previous physiological work we found that Y, or α, cells in retina and the LGN of cats are more tolerant than X, or β, cells during brief elevation of IOP.[15–17,31] This phenomenon in brief IOP elevation conflicts with the morphologic findings reported herein and those reported by others in chronic IOP elevation.[2–4,6–7] Logically, these opposite observations must

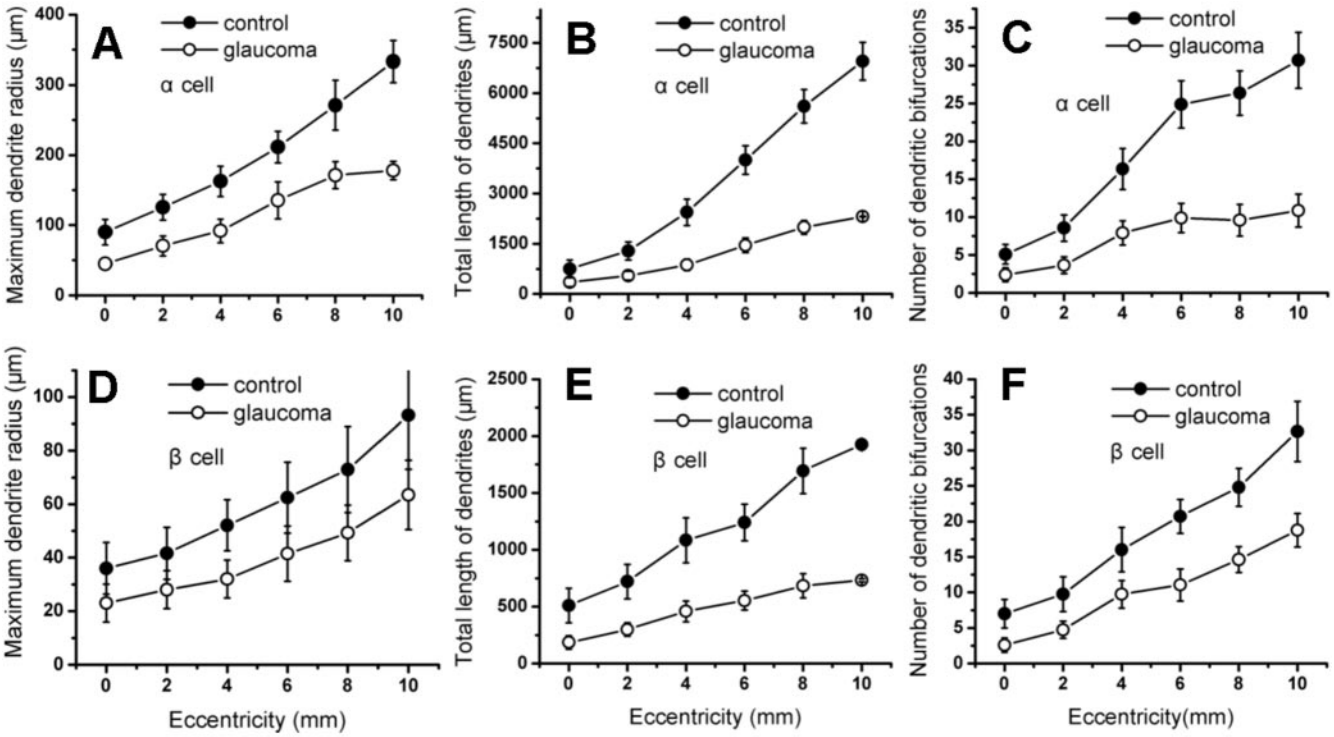

FIGURE 4. Spatial distributions of dendritic structure of α (**A**–**C**) and β (**D**–**F**) RGCs in the normal and glaucomatous eyes. (**A**, **D**) Distributions of maximum dendrite radius. (**B**, **E**) Distributions of the total length of dendrites of each neuron. (**C**, **F**) Distribution of the number of dendritic bifurcations. All the α cells showed significantly greater shrinkage in dendritic structure than the β cells. Data were from one eye of six cats with unilateral elevation of IOP (mean IOP = 31.6 mm Hg) for approximately 2 to 3 weeks. Error bars: SD.

reflect the difference between the acute and chronic effects of IOP elevation. Depending on the strength and duration of pressure, elevation of IOP can cause retinal and optic nerve ischemia, interruption of axoplasmic transport, axonal degeneration, axon degeneration, and cell death in RGCs.[1–6] In the acute physiological experiments, the effects of IOP were reversible because the elevation was short in duration (5–20 minutes), although the increase in IOP was acute (approximately 90 mm Hg, which was approximately 25–30 mm Hg lower than the animal's arterial blood pressure).[15–17] However, in the cats with chronic glaucoma, the duration of IOP elevation was a few weeks, even as long as months, during which the RGCs were severely damaged, although the IOP was relatively low (mean IOP ranged from 24.5 to 39.5 mm Hg). Y, or α, ganglion cells may have more intracellular reserves of adenosine triphosphate (ATP), oxygen, and potassium than X, or β, cells during brief ischemia induced by short-term IOP elevation.[32–35] Thus, Y cells tolerate briefly elevated IOP better than X cells. In contrast, in the eyes with chronic glaucoma, the long-term effects induced by elevated IOP may deprive Y cells of oxygen and ATP more than X cells, because of their large cell size, smaller surface-to-volume ratio, lower response threshold, and higher demand of energy and oxygen. This may explain why α cells died and shrank more significantly than did β cells in the eyes with chronic glaucoma.

The cell-type–differentiated dendritic shrinkage we observed is more sensitive to IOP elevation than soma atrophy, which declined to the same degree in β and α cells. This supports the idea that the RGCs in glaucomatous eyes undergo a process of degeneration that originates with the dendritic arbor, continues with reduction of axon thickness, and ends with shrinkage of the cell body.[7] The earliest signs of the IOP elevation-induced differential effect of degeneration associated with altered dendritic arbor and cell death may provide a good opportunity for neuroprotection in clinical treatment. Thus, an early visual evoked potential measure of contrast sensitivity functions is suggested, using moving grating stimuli of different velocities to the peripheral retina, where more of the motion-sensitive Y cells are lost and injured.

Acknowledgments

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