Morphology of Single Ganglion Cells in the Glaucomatous Primate Retina

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PURPOSE. To examine the degenerative effects that prolonged elevation of intraocular pressure (IOP), a risk factor commonly associated with glaucoma, has on the morphology of single ganglion cells in the primate retina.

METHODS. The monkey model of glaucoma was combined with intracellular staining techniques using an isolated retina preparation. Midget and parasol cells from normal and glaucomatous eyes were labeled intracellularly, and their axons, somas, and dendritic fields were compared using confocal microscopy.

RESULTS. In midget and parasol cells, the earliest signs of pressure-induced degeneration involved structural abnormalities associated with the dendritic arbor. Reductions in axon thickness appeared later, with changes in soma size occurring concomitantly or slightly later. Chronic elevation of IOP resulted in a significant decrease in the mean soma sizes of midget and parasol cells, but only parasol cells showed a significant reduction in dendritic field size and axon diameter. Comparisons of eyes with different levels of optic nerve damage, based on cup-disc ratio, showed that the axons and dendritic fields of parasol cells were significantly smaller at lower cup-disc ratios than were those of midget cells, suggesting a possible differential effect.

CONCLUSIONS. In glaucoma, retinal ganglion cells undergo a pattern of degeneration that originates with the dendritic arbor and ends with shrinkage of the cell soma. Although this pattern of degeneration implies early functional deficits and retinal ganglion cell atrophy that occurs earlier than previously thought, based on ganglion cell loss alone, it also suggests a window of opportunity for effective neuroprotection.

Glaucoma is a disease of the visual system that, in many cases, is characterized by an elevation of intraocular pressure (IOP), progressive changes in the appearance of the optic disc and retinal nerve fiber layer, and visual field defects. Although several investigators have described the degenerative effects that chronic elevation of IOP and glaucoma have on fibers in the optic nerve14-16 and the concomitant loss of ganglion cells that occurs within the retina itself,7-12 the morphologic changes that characterize the atrophy of single ganglion cells in the glaucomatous eye remain unknown. These data are important, because the spatial and temporal processing of visual information by retinal ganglion cells depends on their structural integrity.13,14 In addition, an understanding of the pattern of glaucomatous neuropathy that occurs at the single-cell level will benefit ongoing studies conducted to develop neuroprotectant-based treatment strategies.

Although it is possible to identify, based on soma size and dendritic field architecture, several different types of ganglion cells in the primate retina, the midget and parasol cells have been described most completely.18-29 In brief, midget cells represent approximately 80% of the ganglion cells in the primate retina, they have medium-sized somas, and their small- to medium-sized dendritic trees often originate from a single dendrite that then gives rise to a compact, bushy dendritic arbor. The axons of midget ganglion cells project to the dorsal, parvocellular, layers of the lateral geniculate nucleus (LGN), and they represent the P-pathway of the primate visual system. Functionally, these neurons have small receptive fields and more slowly conducting axons than parasol cells, and they respond best to chromatic stimuli of high spatial and low temporal frequency. Parasol cells represent approximately 10% of the total population of ganglion cells in the primate retina. At all retinal eccentricities, the somas and dendritic fields of these neurons are among the largest in the ganglion cell layer, and their dendritic trees often originate from three to four thick primary dendrites that branch regularly and form a radially symmetrical arbor. The axons of parasol cells typically are larger than the axons of midget ganglion cells. Parasol cells project to the ventral magnocellular layers of the LGN, and they represent the M-pathway of the primate visual system. Functionally, parasol cells have large receptive fields with rapidly conducting axons, and they respond best to achromatic stimuli of high temporal and low spatial frequency.

To examine the morphology of midget and parasol cells in the glaucomatous eye, we combined the monkey model of experimental glaucoma31-34 with intracellular staining tech-
TABLE 1. Summary of Animals Studied

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Mean IOP (mm Hg)*</th>
<th>Peak IOP (mm Hg)</th>
<th>Duration (wk)</th>
<th>Cup/Disc Ratio (initial/final)</th>
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<tr>
<td>M81102</td>
<td>M</td>
<td>11</td>
<td>46 ± 17</td>
<td>62</td>
<td>2.5</td>
<td>0.2/0.9</td>
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<td>AE96</td>
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<td>44 ± 21</td>
<td>58</td>
<td>2.5</td>
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<td>AA02</td>
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<td>18</td>
<td>46 ± 9</td>
<td>72</td>
<td>4</td>
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<tr>
<td>M80005</td>
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<td>8</td>
<td>52 ± 19</td>
<td>80</td>
<td>4</td>
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</tr>
<tr>
<td>W84</td>
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<td>19</td>
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<td>76</td>
<td>8</td>
<td>0.2/0.8</td>
</tr>
<tr>
<td>M80085</td>
<td>M</td>
<td>13</td>
<td>52 ± 12</td>
<td>76</td>
<td>8</td>
<td>0.2/0.8</td>
</tr>
<tr>
<td>M79047</td>
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<td>13</td>
<td>66 ± 25</td>
<td>66</td>
<td>12</td>
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<td>AM92</td>
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<td>M88081</td>
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<td>M80142</td>
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<td>36 ± 9</td>
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<td>M1799†</td>
<td>F</td>
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<td>32 ± 15</td>
<td>66</td>
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<tr>
<td>M1755‡</td>
<td>F</td>
<td>10</td>
<td>24 ± 11</td>
<td>55</td>
<td>49</td>
<td>0.3/0.5</td>
</tr>
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IOP, intraocular pressure.
* Values are means ± SD.
† Laser and microsphere treatment.
‡ Bilateral microsphere treatment.

Subjects and Procedures

Fourteen rhesus monkeys (Macaca mulatta) of both sexes, aged 6 to 19 years, were used in this study (Table 1). All had clinically normal-appearing eyes, determined by slit lamp biomicroscopy, gonioscopy (using a lens by Carl Zeiss, Oberkochen, Germany) and stereoscopic funduscopy (fundus camera by Zeiss or Topcon, Tokyo, Japan) and all had baseline intraocular pressure, measured under ketamine HCl anesthesia with either a tonometer (minifed Goldman35) or (Tono-pen XL; Mentor O&O, Norwell, MA), below 21 mm Hg (normal IOP range of anatomic features for use in the identification of glaucoma-related changes in ganglion cell morphology.

Based on morphologic, topographic, and functional data, the isolated retinas contained a large complement of retinal ganglion cells.

In 12 animals, chronic elevation of IOP and experimental glaucoma were induced by argon laser scarification of the trabecular meshwork. Each animal first was anesthetized with 10 mg/kg ketamine HCl intramuscularly, supplemented with 5 mg/kg as needed, and sedated with 1 mg/kg diazepam intramuscularly. A standard clinical argon laser (model 900; Coherent, Palo Alto, CA) and slit lamp delivery system was used to produce a series of focal lesions to the trabecular meshwork in one eye (50–250 spots, 50-μm spot diameter, 1–1.5 W, 0.5 seconds' duration). Intraocular pressure was monitored for 2 to 3 weeks after treatment and, if not consistently 25 mm Hg or more, additional laser treatments were performed until stable ocular hypertension was achieved. The opposite eye served as a normal control (additional normal eyes obtained through the Wisconsin Regional Primate Research Center Tissue Distribution Program, Madison, WI).

In the remaining two animals, IOP was elevated by injecting 100 μl sterile aqueous solution containing approximately 3.99 × 10^5 latex microspheres (10-μm diameter; Molecular Probes, Eugene, OR) into the anterior chamber of each eye.4 Injection was made using a 30-gauge needle and a transcorneal approach that resulted in little, if any, backflow. Generally, 8 to 10 injections were needed to produce an initial sustained elevation of IOP. The frequency of additional injections, and determination of the volume of beads to be injected, was based on biweekly measurements of intraocular pressure.

Approximately once a week after laser treatment and twice a week after the initial injection of latex microspheres, the normal and treated eyes were examined with a slit lamp (Zeiss), noting corneal clarity and cells and flare in the anterior chamber. After measurement of IOP, the anterior chamber angle and optic nerve head were examined with a goniolens (Zeiss, or OG3M-13; Ocular Instruments, Bellevue, WA). Fundus photographs were obtained approximately every 3 to 4 weeks, depending on the clinical appearance of the optic disc.
compared with its appearance in the previous eye examination.

After periods of elevated IOP that ranged from 2.5 weeks to 49 weeks, the animals were anesthetized deeply with 15 mg/kg ketamine HCl intramuscularly, followed by an intravenous injection of 35 mg/kg pentobarbital sodium. The eyes were removed quickly, and the animal received an overdose of pentobarbital sodium and was perfused transcardially with 0.5 l of 0.9% saline followed by 1 l of 10% formalin solution. The brains were removed and postfixed for future histologic examination of the LGN.35

In Vitro Procedures and Tissue Processing

Immediately on enucleation, the anterior segment of each eye (from the ora serrata forward) was removed with a pair of fine scissors and the posterior eyecup placed in a solution of artificial cerebrospinal fluid (pH 7.4)36 saturated with a mixture of 95% O2 and 5% CO2 at room temperature. The vitreous body was removed and the retina isolated. Care was taken to avoid mechanical stress, especially near the fovea where the retina is very thin. The retina then was flattened and placed ganglion cell layer up in a plexiglass chamber also perfused with 6 ml/min to 8 ml/min oxygenated artificial cerebrospinal fluid. The tissue was held submerged in the chamber by a small nylon net, and the chamber was mounted on the stage of an upright microscope equipped with epifluorescence. A neutral density filter (ND4; Nikon, Tokyo, Japan) was used to reduce the intensity of the mercury vapor light reaching the tissue. Single ganglion cells were viewed using a 40X water immersion objective (numerical aperture 0.55; Nikon) with a working distance of 1.6 mm. Periodically, a few drops of a 1 mM solution of the vital dye acridine orange (catalog no. A-4921; Sigma, St. Louis, MO) was added to the tissue chamber to aid in visualizing single ganglion cells.18-20

Intracellular injections of single retinal ganglion cells were made using glass microelectrodes and a four-axis hydraulic micromanipulator attached to the microscope stage. Glass micropipettes were pulled on a micropipette puller (Flaming/Brown, model P-87; Sutter Instruments, Novato, CA) and filled with a solution of 3% Lucifer yellow CH (catalog no. L-0259; Sigma) in 0.1 M LiCl (pH 7.6). For injection, single retinal ganglion cells were first positioned in the center of the microscopie field and the dye-filled electrode was lowered into position adjacent to the cell body. Each cell then was impaled by slowly advancing the electrode along its axis until the tip of the electrode was seen to penetrate the cell's membrane. Complete filling of the soma, dendritic tree, and from 1 mm to 2 mm of the cell's intraretinal axon segment was achieved by passing a negative current (1-5 nA) through the electrode. Typically, 1 to 2 minutes was sufficient to label completely the dendritic trees of midget and parasol cells. The progress of each intracellular injection was monitored visually, with care taken to minimize the amount of time that individual cells were exposed to the mercury vapor light. After the last cell was injected, the retina was removed from the injection chamber and immersion-fixed in 4.0% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). Each retina then was rinsed with 0.1 M sodium phosphate buffer, whomeloaded on a gelatinized glass slide, dehydrated, defatted, and protected with a coverslip.

Intracellular injections of single retinal ganglion cells were evaluated with a two-tailed Student's t-test. All statistical distributions were compared using the Mann-Whitney test, and differences in mean soma, axon, and dendritic field sizes were evaluated with a two-tailed Student's t-test. All statistical analyses were performed using commercially available statistical software (SPSS, Chicago, IL), with P = 0.05 used as the level of significance.

RESULTS

A total of 1298 intracellularly labeled retinal ganglion cells, judged by visual inspection to be filled completely, were analyzed in this study. Of these, 518 cells (111 midget and 407 parasol) were injected in normal retinas, and 780 ganglion cells (236 midget and 544 parasol), were labeled in glaucomatous eyes.

Qualitative Observations

Parasol Cells. Confocal images of parasol cells that were labeled intracellularly in the normal primate eye are shown in Figure 1. In agreement with previous studies,13-18,20-24 these neurons have large cell bodies; large, radially oriented dendritic trees that originate from three to four primary dendrites; and thick proximal axon segments. In addition, their dendritic processes branch regularly, and they show a progressive decrease in size with increased branching and distance from the cell soma. Although the main dendrites of most normal parasol cells had a relatively smooth appearance, it was not uncommon to see numerous fine dendritic appendages associated with the arbors of these cells.20

Sampling, Classification, Mapping, and Morphologic Analysis of Retinal Ganglion Cells

Because the intracellular approach does not permit the injection of a large number of neurons at every location in each retina studied, we focused our morphologic examination on ganglion cells located in the superior and inferior regions of the midperipheral temporal retina (~2-7 mm, ~10°-35°, from the fovea), the area considered clinically to be most vulnerable to glaucoma-related neuronal damage.3,5,7-9 In addition, we restricted our analysis to only those labeled cells that, based on anatomic comparisons with other intracellular studies in the primate retina,18-20,24,40,41 we considered to be midget and parasol cells.

Because retinal ganglion cell size varies with retinal eccentricity,15-24 the position of each labeled ganglion cell relative to the location of the optic disc and fovea was recorded using a microscope-based digitizing system (Minnesota Datametrics, St. Paul, MN). This retinal map also served as a guide for the reconstruction of each labeled ganglion cell using a confocal microscope (MRC 600 Lasersharp; Bio-Rad, Richmond, CA). Measurements of cell soma, dendritic field area, and axon diameter at a point approximately 5 μm to 10 μm from the cell body, were made directly from the confocal images (projected z-series; resolution, 0.1-0.5 μm/optical section) using the morphometric software of the confocal system. Somal area was determined by tracing the maximum cell body outline of each optically reconstructed neuron, and dendritic field size was obtained by measuring the enclosed area formed by connecting the distal tips of each neuron's dendritic processes.20 Measurements for each experimental group, normal versus glaucoma, were pooled, and all mean data comparisons are presented as means ± 1 SE. Soma and dendritic field size distributions were compared using the Mann-Whitney test, and differences in mean soma, axon, and dendritic field sizes were evaluated with a two-tailed Student's t-test. All statistical analyses were performed using commercially available statistical software (SPSS, Chicago, IL), with P = 0.05 used as the level of significance.
The photomicrographs in Figure 2 outline the range of morphologic features characteristic of parasol cells from glaucomatous eyes. The cells shown are from six animals, and they are arranged based on increasing C-D ratios for the eyes from which they were obtained. The cell in Figure 2A was injected in the superior-temporal retina (~6.6 mm from the fovea) of an eye that had had a mean IOP of 36 mm Hg for 16 weeks. At the time of death, the nasal and temporal regions of the disc showed normal color, the C-D ratio had increased from 0.3 to 0.6, and the disc was slightly more cupped superiorly than inferiorly. Despite these changes, however, the soma, dendritic field, and axon (arrowhead) of this cell are normal in size and appearance for parasol cells at this retinal location. The cell in Figure 2B also was injected in the superior-temporal retina of
an eye that had had the pressure elevated for 16 weeks, but in this eye the mean IOP was only 26 mm Hg. Although the disc was pink at death, the C-D ratio had nearly doubled (0.5 versus 0.3). Although the soma, axon, and dendritic arbor of this neuron also were normal in size, its dendritic processes showed more variability in thickness (arrows) than in the normal cells (Fig. 1) or in the neuron shown in Figure 2A. The parasol cell in Figure 2C was injected in the superior-temporal retina (~3.4 mm from the fovea) of an eye that had had a mean IOP of 46 mm Hg for 1 month. At the time of death the disc had
good color but was deeply cupped, having progressed from a C-D ratio of 0.3 to 0.8. In addition, there was a small hemorrhage in the superior-temporal region of the disc. Although the cell body, axon, and dendritic tree of this neuron are normal in size for its retinal location, the proximal portion of one primary dendrite (arrow) is abnormally thin compared with its distal segment. In addition the cell body does not have the smooth, round shape exhibited by the other parasol cells. The cell in Figure 2D, similar to that in Figure 2A, was injected in the superior-temporal region of an eye that had had a mean IOP of 37 mm Hg for 16 weeks. Unlike the cell in Figure 2A, however, the C-D ratio of the eye from which this cell was recovered had increased from a baseline value of 0.3 to 0.8 at the time of death, and the temporal region of the disc was paler than the nasal region. Although the cell’s overall appearance was not abnormal, some of its higher order dendrites were thinner than their parent processes (arrows). The axon of this neuron also could be seen to undergo an abrupt decrease in thickness (arrowheads). The parasol cells shown in Figures 2E and 2F are from eyes with C-D ratios of 0.9. The cell in Figure 2E was injected in the superior-temporal retina (~4.5 mm from the fovea) of an eye with an IOP averaging 46 mm Hg for only 2.5 weeks. At the time of death, the nerve head was deeply cupped, and the temporal region of the disc was pale. Although the size and general organization of the cell’s dendritic field are similar to those of normal parasol cells at this retinal location, several structural abnormalities can be seen. In particular, unlike normal parasol cells (Fig. 1), the dendrites of this neuron did not show a progressive decrease in size with increased branching and distance from the cell soma. Instead, most of this neuron’s primary dendrites were uniformly large and clublike, undergoing abrupt changes in diameter at distal branch points and giving rise to thin, wispy terminal dendritic processes. In addition, although the cell displayed a normal, spherical-shaped soma, the cell body was smaller than that of most normal parasol cells found at this retinal location and smaller than that of cells containing a dendritic arbor of comparable size. Further, the cell’s proximal axon (arrowhead) was abnormally thin, measuring only half the mean thickness of axons of normal parasol cells with comparable-sized somas (0.75 μm versus 1.5 μm). The parasol cell in Figure 2F is from the superior-temporal retina of a glaucomatous eye that had had a mean IOP of 42 mm Hg for 8 weeks. At time of death, the eye had a cup/disc ratio of 0.9, the disc was deeply cupped, and the temporal region of the disc was paler than the nasal region. Although the somas, dendritic arbor, and proximal axon (arrowhead) of this neuron were normal in size for its retinal location (~5.5 mm from the fovea), the primary dendrites of this neuron were abnormally thin, and the distal dendritic processes showed considerable variability in thickness.

**Midget Cells.** Examples of normal midget ganglion cells are shown in the confocal images in Figure 3. In contrast with normal parasol cells (Fig. 1), these neurons were characterized by their small cell bodies, smaller and more compact dendritic arbor, and relatively thin proximal axon segments. In addition, the dendritic trees of most midget cells originate from a single primary dendrite, and they typically are not radially symmetrical around the cell body. Further, the dendritic arbors of midget ganglion cells contain finer processes that are more variable in size and length than are the dendritic processes of parasol cells.13-24

The confocal images in Figure 4 show the range of morphology associated with midget ganglion cells injected in the temporal retinas of eyes with glaucoma. The cells shown were from four different eyes and again were arranged based on increasing C-D ratios for their respective eyes. The midget cells in Figures 4A and 4B were injected in the inferior-temporal retina of the same eye as the parasol cell shown in Figure 2A (mean IOP, 36 mm Hg for 16 weeks; C-D, 0.6). Similar to the parasol cell, the somas, dendritic arbors, and axons (arrowheads) of these neurons were normal in size and appearance for midget cells at this retinal location (~6.8 mm from the fovea). The midget cells in Figures 4C and 4D were injected in the inferior-temporal retina of an eye that had had a mean IOP of 54 mm Hg for 8 weeks. At time of death, the disc was deeply cupped (C-D, 0.8), but showed good color. Although both neurons displayed considerable variability in the thickness of their dendritic appendages, their somas, dendritic fields, and axons were normal in size and appearance.

The midget cell in Figure 4E is from the inferior-temporal retina of an eye that had a mean IOP of 27 mm Hg for approximately 9 weeks. Despite the relatively modest IOP elevation, at time of death the C-D ratio had increased from 0.2 to 1.0, there was undermining of the rim superiorly, and the temporal region of the disc was very pale. Although the soma of this neuron was comparable in size with that of the normal midget cell shown in Figure 3A, its dendritic arbor was highly abnormal, being reduced to only a few thin, sparsely branched processes. In addition, the axon was very thin compared with that of other midget cells of comparable soma size. Similar to the cell in Figure 4E, the midget ganglion cell shown in Figure 4F was from a glaucomatous eye that had a final C-D ratio of 0.9. This cell, however, was injected in the superior-temporal retina of an eye that had a mean IOP of 44 mm Hg for only 2.5 weeks. Although the cell’s general morphology was similar to that of the normal midget cell shown in Figure 3A, its dendritic arbor was extremely abnormal, being reduced to only a few thin, sparsely branched processes. Although the soma and dendritic field of this neuron were within the normal size range, they were smaller than most midget ganglion cells at this retinal location, as was the diameter of its axon.

**Quantitative Observations**

**Ganglion Cell Soma Sizes in Normal and Glaucomatosous Eyes. Parasol Cells.** The histograms in Figure 5A illustrate the distributions of parasol cell soma cross-sectional areas sampled in the midtemporal retinas of the normal (upper histogram) and glaucomatous (lower histogram) eyes studied. Although the normal parasol cells had somas that ranged from 75 μm² to 500 μm², approximately 90% of these neurons had somas that were within the narrower range of 100 μm² to 350 μm² consistent with findings in previous studies of normal parasol cells in this area of the retina.25 Similarly, parasol cells injected in glaucomatous eyes had somas that ranged from 50 μm² to 550 μm², with 80% having cross-sectional areas between 75 μm² and 300 μm². However, in proportion to the total number of ganglion cells, relatively more parasol cells with somas 200 μm² or less were found in the retinas of glaucomatous eyes than in normal eyes. This resulted in a skewed distribution of glaucoma-related parasol cell soma sizes, and a statistical difference (P < 0.001) from the distribution of normal parasol cell soma sizes. The mean soma sizes
of parasol cells in normal and glaucomatous eyes (Fig. 5B) also were significantly different ($P < 0.001$), with parasol cells in the glaucomatous eyes being approximately 13% smaller than normal. When compared at different retinal eccentricities (Fig. 5C), the mean soma sizes of parasol cells in glaucomatous eyes were consistently smaller than those of normal cells. This reduction in cell body size was significant for parasol cells located 4 mm to 5 mm (23.3%) and 5 mm to 6 mm (15.7%) from the fovea (4-5 mm: $P < 0.001$; 5-6 mm: $P < 0.01$). The histograms in Figure 5D compare the mean soma sizes of parasol cells from normal eyes with cells from glaucomatous eyes that have different C-D ratios. In all cases, the glaucomatous eyes had baseline C-D ratios of 0.3 or less (Table 1). Although parasol cells from eyes considered to be in the early stage of the disease process (C-D, 0.4-0.6) had somas that were actually slightly larger (7.5%) than normal ($P < 0.01$),

**FIGURE 3.** (A through F) Confocal images showing the range of morphologic features characteristic of normal midget ganglion cells. Arrowheads indicate intraretinal axon segments. Scale bars, 10 μm.
FIGURE 4. Confocal images showing the morphologic changes that characterize midget ganglion cells from eyes with different levels of optic nerve damage. (A, B) Mild; (C, D) moderate; (E, F) severe. Arrowheads indicate intraretinal axon segments. Scale bars, 10 μm.

cells from eyes with C–D ratios of 0.6–0.8 were not different from normal cells, and cells in eyes with C–D ratios more than 0.8 were significantly smaller (43.2%) than normal (P < 0.001).

Midget Cells. Most of the midget ganglion cell somas in normal eyes (Fig. 6A, upper) had the same size range as that of midget cells injected in glaucomatous eyes (Fig. 6A, lower), with approximately 80% to 85% of the cells in each group having cross-sectional areas that ranged from 75 μm² to 200 μm², again consistent with findings in studies of midget ganglion cells in this area of the retina. Although the distribution of soma sizes for midget cells in the glaucomatous eye was slightly broader than that for normal midget cells, the two distributions were not significantly different (P = 0.09). Nevertheless, the mean soma sizes of midget ganglion cells in the glaucomatous eye were significantly smaller (12.5%; P < 0.01) than those of midget cells in the normal eye (Fig. 6B). This
Figure 5. Soma size comparisons for parasol cells from normal (open) and glaucomatous (batched) eyes. In general, the somas of parasol cells from glaucomatous eyes were significantly smaller than normal (A, B). This difference was most pronounced in cells in the midperipheral temporal retina (C) and in those from eyes with severely cupped (cup–disc, 0.8–1.0) optic discs (D).

Ganglion Cell Dendritic Field Sizes in Normal and Glaucomatous Eyes. Parasol Cells. A comparison of the size distributions of the dendritic fields of parasol cells from normal and glaucomatous eyes is shown in Figure 7A. Similar to parasol cell somas (Fig. 5A), the mode of the distribution representing the dendritic field sizes of parasol cells from glaucomatous eyes was shifted toward sizes smaller than those of normal parasol cells, and the distributions were statistically different ($P < 0.001$). Similarly, the mean dendritic field sizes of parasol cells from glaucomatous eyes were significantly smaller (14.7%; $P < 0.001$) than those of neurons sampled from comparable regions of normal retinas (Fig. 7B). This difference was most consistent and significant in cells located farther than 4 mm from the fovea (Fig. 7C). Compared across eyes with different C-D ratios (Fig. 7D), the dendritic fields of parasol cells in
glaucomatous eyes were 24.9\% smaller than normal in eyes with C-D ratios of 0.6 to 0.8 and 42.4\% smaller than normal in eyes with C-D ratios more than 0.8 (both: \( P < 0.001 \)).

**Midget Cells.** The size distributions of the dendritic arbors of midget ganglion cells in normal and glaucomatous monkeys are shown in Figure 8A. As noted by others, and shown in the photomicrographs of Figures 1 to 4, the dendritic arbors of midget ganglion cells in the primate retina were much more compact than those of parasol cells. This morphologic difference was reflected in the nearly 10-fold difference in the size distributions for these two classes of neurons (Fig. 8A versus Fig. 7A; note scales). Although more than 75\% of the midget cells sampled in normal and glaucomatous eyes had dendritic fields less than 200 \( \mu \text{m}^2 \) in area, cells with arbors as large as 4000 \( \mu \text{m}^2 \) were measured in both groups of eyes.\(^9\)\(^9\) No significant differences were found between the dendritic field size distributions or the mean dendritic field sizes (Fig. 8B), of midget ganglion cells injected in normal and glaucomatous eyes (\( P > 0.5 \) for both). Unlike parasol cells, no consistent or significant difference was found in the dendritic field sizes of midget cells within the range of retinal eccentricities studied (Fig. 8C). As with soma size, the dendritic fields of midget ganglion cells from eyes with C-D ratios less than 0.8 were not different from normal, whereas those from eyes with severe...
Figure 7. Dendritic field size comparisons for intracellularly labeled parasol cells from normal (open) and glaucomatous (hatched) eyes. In agreement with their smaller soma sizes, the dendritic fields of neurons from glaucomatous eyes were smaller than normal (A, B). This difference was most pronounced in the peripheral retina (C). Unlike soma size, the dendritic fields of parasol cells from glaucomatous eyes were significantly smaller than normal in eyes with moderate (cup-disc, 0.6–0.8) and severe (cup-disc, 0.8–1.0) levels of optic disc cupping (D).

Axon Thickness of Midget and Parasol Cells in Normal and Glaucomatous Eyes. Proximal axon thickness in all midget cells ranged from 0.2 μm to 2.5 μm, with a mean size of 1.0 μm ± 0.04 (±SE; N = 227; Fig. 9A). In agreement with their larger cell body sizes, parasol cells from normal and glaucomatous eyes showed the largest axon segments (Fig. 9B). In normal eyes, parasol cell axon diameters ranged from 0.8 μm to 4.2 μm, with a mean diameter of 1.6 ± 0.03 μm (N = 390). In glaucomatous eyes, the initial segments of parasol cell axons tended to be smaller (0.5–3.2 μm), with a mean diameter of 1.4 ± 0.05 μm (N = 509). Only the difference in the mean axon diameters of parasol cells was significant (P < 0.001). Comparisons of axon diameter in ganglion cells with different C-D ratios (Figs. 9C, 9D) produced results similar to those seen in comparisons of soma and dendritic field sizes. Although the size of axons of parasol cells from eyes with C-D ratios of 0.4 to 0.6 were not different from normal, the axons of parasol cells from eyes with C-D ratios of 0.6 to 0.8 were...
FIGURE 8. Comparisons of the dendritic field sizes of midget ganglion cells labeled in normal (open) and glaucomatous (hatched) eyes (A). Unlike parasol cells, the dendritic fields of midget cells were not significantly different from normal (B, C), except in eyes with severe (cup-disc, 0.8-1.0) glaucomatous cupping of the optic nerve head (D).

12.5% smaller than normal ($P < 0.001$), and those from eyes with C-D ratios more than 0.8 were 43.8% smaller than normal ($P < 0.001$). Similar to the pattern seen in dendritic field measurements, only the axons of midget ganglion cells from eyes with C-D ratios more than 0.8 were significantly smaller (40%) than normal ($P < 0.001$).

**DISCUSSION**

In this study, we used intracellular staining techniques to examine the degenerative effects that chronic elevation of intraocular pressure, a risk factor commonly associated with glaucoma, has on the morphology of single ganglion cells in the primate retina. The data show that in midget and parasol cells, the earliest structural signs of glaucomatous neuropathy involved changes at the level of the dendritic arbor. These changes included a thinning of the proximal and distal dendrites, abrupt reductions in dendritic process diameter at branch points, and a general decrease in the complexity of the cell's dendritic tree. Decreases in axon diameter seemed to occur later than changes at the level of the dendritic arbor, whereas a reduction in soma size occurred concurrently or slightly later.

Comparisons of ganglion cells from eyes with different levels of optic nerve damage, based on C-D ratios, suggested...
that despite qualitative changes in appearance, the sizes of the somas, dendritic fields, and axons of midget and parasol cells typically were not reduced significantly with a C-D ratio of approximately 0.6 or more. This finding agrees closely with previous reports that the probability of glaucoma-related abnormalities increases significantly when the C-D ratio equals or exceeds 0.6.\textsuperscript{39} However, it is important to note that in most cases, our final C-D ratio assessments were made on eyes with elevated levels of IOP (47.6 ± 15 mm Hg, on average). Because the optic disc of the young monkey is highly compliant and therefore can undergo pressure-induced deformations that may or may not be reversible,\textsuperscript{42} the possibility exists that we overestimated slightly our C-D ratios, and therefore the level of optic nerve damage, in the glaucomatous eyes. That the optic discs of eyes with C-D ratios even in the range of 0.8 to 1.0 showed good color across most of the disc area, with pallor restricted primarily to the temporal regions, supports this possibility. An overestimation of C-D ratio, however, implies that the neuronal changes we describe occurred at an even earlier stage of the disease process.

**Identification of Pressure-Induced Degeneration**

Because the data presented here were obtained using an in vitro preparation, it can be argued that many of the morphologic changes ascribed to elevated IOP resulted instead from severing the axons of ganglion cells during isolation of the retina. This seems unlikely for several reasons. First, the dendritic abnormalities described in Figures 2 and 4 were uncharacteristic of normal midget and parasol cells examined using the same experimental conditions. Second, we were unable to...
induce comparable structural changes in normal ganglion cells by altering parameters associated with the in vitro preparation or with our intracellular staining techniques. Reducing the flow of oxygenated solution to the tissue, exposing single neurons to the mercury vapor light for extended periods (>15 seconds), or staining cells using high levels of negative current (>5 nA), resulted in neurons whose axons and dendrites contained numerous large, regularly spaced translucent varicosities. These structures were atypical of ganglion cells from normal and glaucomatous eyes using our standard experimental procedures. Although results of previous Golgi and retrograde labeling studies have suggested that dendritic beading may be a regular feature of primate ganglion cells, Roof et al. our data do not support this suggestion and are consistent with data in other studies in which similar intracellular staining techniques have been used to examine the morphology of midget and parasol cells in the primate retina. Thus, although we cannot rule out the possibility that some neurons were affected adversely by our experimental approach, we feel confident that the morphologic abnormalities we have described for ganglion cells in the glaucomatous eye are highly representative of degenerative changes caused by elevated IOP and not by the in vitro technique per se.

**Midget Versus Parasol Cells**

An issue common to many studies of glaucomatous retinopathy is whether midget and parasol cells, and therefore the P- and M-pathways of the primate visual system, are affected differentially by the disease. The significance of this issue derives from the fact that these pathways are considered to subserve different functional roles, and thus a preferential effect on one or the other could have important implications for the development of more sensitive psychophysical tests to achieve early detection of the disease. In recent studies in which changes in axon diameter, soma size, and neurofilament content have been examined in ganglion cells from eyes with glaucoma, results have indicated that although cells of all sizes are lost, large ganglion cells appear to be affected most severely. That these large neurons may represent primarily parasol cells and the M-pathway is suggested by data indicating an apparent differential effect on neurons in the magnocellular and parvocellular layers of the LGN. Functional support for a preferential effect on the M-pathway also has been provided by psychophysical studies showing a diminution in pattern electroretinogram and pattern visual evoked responses to stimuli of low spatial and high temporal frequencies and a reduction in motion detection. In the glaucomatous eye.

Although the axon and dendritic field size measurements presented here also indicate that chronic elevation of IOP may have a more detrimental effect on parasol than midget ganglion cells, the data, in general, suggest that the magnitude of any differential effect may be small. Midget and parasol cells showed similar patterns and degrees of degenerative change, and there was no clear indication in any of the retinas examined that a disproportionate number of cells representing either class were more affected. In addition, the somas of midget and parasol cells were found to undergo similar reductions in size (12.5% and 13.3%, respectively), and in both classes of neurons these reductions were significant only in eyes with advanced glaucomatous damage. Both classes of cells showed similar reductions in dendritic field size (midget, 41.3%; parasol, 42.4%) at this stage of the disease as well. That a reduction in soma size was not prevalent during mild and moderate stages of the disease is in agreement with Glovinsky et al., who concluded that the differences in cell size distributions between normal and glaucomatous monkey eyes are not caused by selective shrinkage of large ganglion cells. Recent studies in which the rat model of glaucoma has been used, however, have indicated that the somas and dendritic fields of retinal ganglion cells in the glaucomatous eye may actually increase in size as a result of the disease process. Although we did not find the somas or dendritic fields of midget or parasol cells as a whole to be significantly larger than normal in any of our experimental eyes, it is interesting to note that the comparisons of soma and dendritic field size versus C-D ratio showed small increases in these features in eyes with mild glaucoma. Whether this represents a short-term initial response by these neurons to the effects of elevated IOP or is simply a slight sampling bias remains unclear.

The subtle differences in the degenerative patterns of midget and parasol cells reported here do not exclude the possibility of a true differential effect. Although the intracellular method provided detailed information concerning the soma, axon, and dendritic morphologies of single ganglion cells, it was limited in the range of retinal area and number of ganglion cells that could be examined in a single eye. Because the region of retinal ganglion cell loss in glaucoma can be highly variable, it is likely that by restricting our injections to the midtemporal retina our cell samples were not always focused on the most severely affected area of each retina. Similarly, the pattern of axon and ganglion cell loss in glaucoma can be diffuse, not all cells within the sample region were necessarily affected equally. Indeed, with the exception of eyes that had uniformly pale, deeply cupped optic discs, with near complete loss of ganglion cells in midtemporal retina, a wide variety of cell morphology could be found in each glaucomatous eye. For this reason, it is important to keep in mind that although the qualitative data presented here show the range of morphologic features characteristic of ganglion cells from eyes with different levels of glaucoma, the quantitative data reflect soma, dendritic field, and axonal differences in the population as a whole.

A third factor that may have affected our ability to detect a clear differential effect in glaucomatous changes in midget and parasol cells is an unconscious bias toward the injection of neurons with larger cell bodies. In all retinas, these neurons were more visible and easier to inject, particularly in regions close to the fovea. Because previous studies have suggested that cell size, and not cell class, may be the determining factor underlying glaucoma-related retinal ganglion cell degeneration, such a bias may have resulted in the undersampling of small cells unaffected by elevated IOP.

**Pattern of Retinal Ganglion Cell Degeneration**

Our primary goal in carrying out this study was to define more clearly the degenerative effect that chronic elevation of IOP has on the morphology of single ganglion cells in the primate retina. To this end, the data show for the first time that the earliest signs of retinal ganglion cell degeneration in the glaucomatous eye involved abnormalities in the dendritic field structures of these neurons. That these changes preceded anatomic changes at the level of the cell soma indicates that the onset of retinal ganglion cell degeneration in glaucoma oc-
curred earlier than previously thought, based on estimates of ganglion cell loss alone.

Changes in axon diameter also followed those at the level of the dendritic tree, and these seemed to be closely related to changes in cell soma size. This pattern of degeneration is not surprising. Pressure-induced damage to the optic nerve in the region of the lamina cribrosa has been shown to result in a reduction or cessation in the normal flow of intracellular materials between the retinal ganglion cells and their target neurons in the LGN. As a first response to this injury, the retinal ganglion cells begin to pare their distal dendrites to conserve energy and maintain homeostasis at the level of the cell soma. With time, many of the damaged axons undergo retrograde degeneration, further depriving their parent neurons of any residual stores of trophic materials located within the axon. As these stores are depleted, the dendritic field becomes progressively more degenerate, and finally the cell itself begins to shrink. At some as yet undefined point in the degeneration process, an intracellular signal is activated, and the injured neuron undergoes apoptosis. The fact that structural changes at the level of the cell soma are not a primary feature of the degenerative process suggests a window of opportunity for possibly mitigating or reversing glaucomatous neuropathy, possibly through the application of different neuroprotectants or through genetic modification. Investigators have recently identified a wide variety of trophic factors in the developing vertebrate retina, and many of these factors also tend to be similar in size to those of surrounding parasol, and not midget, cells supports the theory that retinal ganglion cell sensitivity in glaucoma may be based primarily on cell size, rather than on cell class. However, it is unlikely that selective atrophy of these two types of neurons alone represents the earliest degenerative changes in glaucoma, in that psychophysical studies also have described defects in the red-green system early in the disease process and this information is thought to be carried by midget ganglion cells.

The relation between dendritic field structure and spatial, temporal, and spectral response abnormalities of midget and parasol cells is not surprising, considering that the spatial, temporal, and chromatic properties of these neurons are derived, in large part, by the number, pattern, and type of synaptic inputs they receive on their dendritic arbors. Ongoing studies are intended to provide better understanding at the single-cell level of the relation between the dendritic field integrity of midget and parasol cells and their spatial and temporal response properties.

Functional Considerations

Our finding that the earliest signs of retinal ganglion cell degeneration in eyes with chronic elevation of IOP involves structural changes associated with midget and parasol cells is consistent with recent psychophysical studies that have described functional deficits related to the M- and P-pathways in glaucoma. In brief, motion-automated perimetry and high-frequency temporal flicker perimetry tests designed to assess the functional integrity of the M-pathway based on the use of moving targets and gratings of low spatial and high temporal frequency—have been shown to be sensitive methods for detecting early visual defects in patients with glaucoma. More recently, Johnson and Samuels have shown that frequency-doubled perimetry also provides a quick and efficient means of screening for glaucomatous visual field loss. The test is based on the phenomenon that when a sinusoidal grating of low spatial frequency (e.g., 0.25 cycles/degree) is presented at a high counterphase rate (>16 Hz), the grating appears to have twice the actual spatial frequency. This doubling illusion, first described by Kelly, is thought to result from the nonlinear summation properties characteristic of neurons in the M-, but not P-, visual pathway.

Of the more recent psychophysical tests intended for detection of early glaucomatous damage and its relation to functional defects in the P-pathway, short wavelength automated perimetry has received the most attention. This test uses large blue targets presented on a bright yellow background to isolate and measure the responsiveness of short wavelength-sensitive mechanisms. To date, short wavelength automated perimetry has proved beneficial not only in the detection of glaucomatous-related deficits, but also in the prediction of glaucomatous damage in eyes with ocular hypertension. Although the blue-sensitive pathway seems to originate from a population of ganglion cells (small-field bistratified) that are structurally and functionally distinct from midget and parasol cells, the axons of these neurons terminate in the parvocellular region (although in the interlaminar zones of the LGN suggests that they are, in general, part of the P-pathway. That the somas and, presumably, axons of these cells tend to be similar in size to those of surrounding parasol, and not midget, cells supports the theory that retinal ganglion cell sensitivity in glaucoma may be based primarily on cell size, rather than on cell class. However, it is unlikely that selective atrophy of these two types of neurons alone represents the earliest degenerative changes in glaucoma, in that psychophysical studies also have described defects in the red-green system early in the disease process and this information is thought to be carried by midget ganglion cells.

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