

# Number of Ganglion Cells in Glaucoma Eyes Compared with Threshold Visual Field Tests in the Same Persons

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**PURPOSE.** To compare the number of retinal ganglion cells (RGCs) topographically mapped with specific visual field threshold test data in the same eyes among glaucoma patients.

**METHODS.** Seventeen eyes of 13 persons with well-documented glaucoma histories and Humphrey threshold visual field tests (San Leandro, CA) were obtained from eye banks. RGC number was estimated by histologic counts of retinal sections and by counts of remaining axons in the optic nerves. The locations of the retinal samples corresponded to specific test points in the visual field. The data for glaucoma patients were compared with 17 eyes of 17 persons who were group matched for age, had no ocular history, and had normal eyes by histologic examination.

**RESULTS.** The mean RGC loss for the entire retina averaged 10.2%, indicating that many eyes had early glaucoma damage. RGC body loss averaged 35.7% in eyes with corrected pattern SD probability less than 0.5%. When upper to lower retina RGC counts were compared with their corresponding visual field data within each eye, a 5-dB loss in sensitivity was associated with 25% RGC loss. For individual points that were abnormal at a probability less than 0.5%, the mean RGC loss was 29%. In control eyes, the loss of RGCs with age was estimated as 7205 cells per year in persons between 55 and 95 years of age. In optic nerves from glaucoma subjects, smaller axons were significantly more likely to be present than larger axons ( $R^2 = 0.78$ ,  $P < 0.001$ ).

**CONCLUSIONS.** At least 25% to 35% RGC loss is associated with statistical abnormalities in automated visual field testing. In addition, these data corroborate previous findings that RGCs with larger diameter axons preferentially die in glaucoma. (*Invest Ophthalmol Vis Sci.* 2000;41:741-748)

Glaucoma is detected and monitored by observations of the state of the optic disc and nerve fiber layer and by tests of the visual field that use light sense perimetry in automated instruments. The extent of damage indicated by these clinical tests must be known to plan appropriate management. For some years, we have collected eyes donated to eye banks from persons known to have glaucoma to compare their histologic features with clinical parameters.

Four previous reports have compared the number of remaining RGCs to the visual field findings in the same eyes by manual, static, and kinetic testing on the Goldmann perimeter.<sup>1-4</sup> In most persons in whom defects were detectable, there was considerable RGC loss. Among 10 eyes of seven persons with suspected glaucoma with normal field test results on the Goldmann perimeter, 7 were more than 2 SD below the normal mean RGC axon number for the whole optic nerve, and 3 were 1 SD below the normal RGC axon number. Eyes with normal manual field tests results varied from 12% to 53% RGC loss. It is not possible to estimate the degree to which these

eyes are representative of all eyes with ocular hypertension. Another report detected no histologic RGC loss in five persons with normal visual fields (two of these were tested by automated perimetry).<sup>5</sup> Many eyes with ocular hypertension would be expected to have no RGC damage; however, that some with suspected glaucoma have statistically significant injury suggests that damage can occur before detection.

Visual field testing is now conducted with automated perimeters and standard thresholding algorithms. In histologic RGC counts from three eyes that had undergone Octopus (two eyes; Interzeag, Haag-Streit Services, Mason, OH) or Humphrey (one eye; San Leandro, CA) automated tests, a considerable number of RGC bodies were dead at a given retinal location before detectable abnormality in a visual field test.<sup>4</sup> Furthermore, these and other data from similar material in human and monkey eyes with experimental glaucoma indicated that RGC loss in early glaucoma was selectively greater among larger ganglion cells,<sup>1,3,4,6-12</sup> although all RGC sizes were affected.<sup>13-14</sup>

There are at least two explanations for RGC death before detectable field loss. First, there is considerable redundancy in the visual system. A stimulus projected onto a particular retinal location affects many RGCs, whose responses would depend on the normal density of their functional areas and the type of stimulus.<sup>15</sup> Even when some RGCs are dead, others subserving the same area could signal the presence of the target. Second, there is considerable variability in psychophysical testing, as well as substantial variation in the responses of normal persons. These variations generate broad limits within which abnormal responses from a subject with glaucoma would be masked until they significantly exceeded the normal range.

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This study provided extensive correlations between the degree of RGC loss and the automated visual field tests in eyes of individuals with glaucoma. In addition, the proportion of RGCs of various axonal diameters was compared with the extent of loss to re-examine the hypothesis that larger cells are more susceptible to injury.

## METHODS

### Acquisition and Selection of Eyes with Glaucoma and Control Eyes

Human eyes were obtained from eye banks and autopsy services (National Disease Research Interchange, Glaucoma Research Foundation, Johns Hopkins Hospital Department of Pathology, Baltimore, MD). This research was approved by the Joint Committee on Clinical Investigation, Johns Hopkins University School of Medicine, and followed the tenets of the Declaration of Helsinki for research involving human subjects. More than 100 pairs of human eyes were obtained that had been identified as having a history of glaucoma. The name of the ophthalmologist or optometrist who had cared for the deceased was obtained, along with permission to receive the clinical information that might be available. Useful information was received in fewer than half the persons, and in only 25% did it confirm that the subject had either ocular hypertension or glaucomatous optic nerve damage. In 18 persons, we received at least one threshold visual field test with the Humphrey perimeter that had been performed within the last 2 years of life, and tests from 13 persons (17 eyes) were usable from eyes with adequate histologic preservation. There were test results ranging from normal to severe damage. Because these specimens are difficult to obtain, we included both eyes of some persons in certain analyses. Because of possible intra-subject correlation of data, we have also presented only one randomly selected eye per subject for critical analyses.

Approximately 50 control human eye bank eyes were obtained through similar sources from donors with no ocular history. After gross inspection of the retina and anterior segment at the dissecting microscope to eliminate those eyes with visible disease, light microscopic evaluation of the retina and optic nerve was conducted to rule out detectable ocular disorders that would affect RGC number. In addition, we required excellent preservation of control and glaucoma tissues, judged by light microscopic examinations before acceptance of each eye for the study.

### Characteristics of Visual Field Data

In 15 fields (12 persons), we obtained the Statpac 1 (Humphrey) analysis, including the sensitivity in decibels, difference from age-normal value, probability of the sensitivity's falling within the normal range (total deviation), and global indices. Only three of the eyes had had a Statpac 2 analysis, including the Humphrey Glaucoma Hemifield Test. We were unable to obtain from the manufacturer the normative data with which to calculate this parameter on the other eyes. All fields satisfied the Statpac level of reliability for false-positive errors, and all had fewer than 33% fixation losses. Because the levels of false-negative errors exceeding Humphrey limits can be seen in reliable subjects with substantial glaucoma injury, no limit was placed on false-negative errors. In each case, the field test used

was the final test before death, unless the final test did not meet the criteria for reliability.

### Preparation of Ocular Tissues

Each of the eyes had been fixed within 24 hours of death (most within 12 hours) in aldehyde fixative (Tables 1, 2, and 3), and the time from death to fixation did not differ significantly between normal and glaucoma-affected eyes. The normal and glaucomatous eyes were group-matched for age, race, and gender. The retina was separated at the ora serrata and optic nerve, and relaxing incisions were made to produce a flat preparation. The position of the optic disc, fovea, and retinal blood vessels were used for orientation. Measurements were made with a caliper to approximate the positions on the retina that corresponded to the locations for 28 selected test points in the Humphrey 24-2 program (Fig. 1). Because there is a high correlation among adjacent test points, the selected locations were spaced throughout the field and included at least two test points from each Humphrey cluster in the Glaucoma Hemifield Test.

We estimated that 3.5° of visual angle was equal to 1 mm on the retina.<sup>16</sup> Centered on each of the 28 test position locations, a piece of retina 1.5 mm in diameter was trephined and held in modified Swinney filter holders (VWR, West Chester, PA) during processing to keep the retina flat. The retina samples were then embedded in resin (JB-4; Polysciences Inc., Warrington, PA), sectioned at 1- $\mu$ m thickness, and stained with 0.1% thionine. Although control eyes had not undergone visual field testing, the same retinal locations were trephined and embedded for comparison to glaucomatous eyes. Sections were cut to show the retinal layers from internal limiting membrane to photoreceptors. Three different pieces of the trephined retina were measured (Image, Ver. 1.47; NIH, Bethesda, MD) before and after processing and embedding in resin. This allowed the determination that average shrinkage was 19%. Because this was accounted for in our calculations of retinal distance and would not differ between control and glaucoma specimens, no further correction was applied for data as presented.

### Quantitative Analysis of Specimens

The number of RGCs was estimated from at least four retinal sections from each retinal position that corresponded to a field test point. Among cells in the ganglion cell layer, those that satisfied the following characteristics were included as presumed RGCs<sup>15</sup>: round or oval cell outline, round or oval nucleus, and cell diameter greater than 7  $\mu$ m. Section length was measured, and the data were expressed as cells per millimeter retinal length (density). The four sections were averaged to give a mean density for each location. The density of glauco-

TABLE 1. Demographic Data on Control and Glaucoma-Affected Subjects

	Control	Glaucoma
Number	17 eyes/17 persons	17 eyes/13 persons
Mean age	76.4 $\pm$ 11.0 y	72.2 $\pm$ 9.3 y
Death-fixation (range)	7.1 $\pm$ 6.0 h (2.5-24 h)	4.7 $\pm$ 3.8 h (1-13.5 h)
Gender	65% male ( <i>n</i> = 11)	69% male ( <i>n</i> = 9)
Race	16 white, 1 black	7 white, 1 black, 5 unknown

TABLE 2. Visual Field Data from Subjects with Glaucoma

	Number	Mean	Range
Mean			
deviation	15 eyes	$-6.43 \pm 4.33$	0.78 to $-15.60$
CPSD	13 eyes	$4.47 \pm 3.88$	0.80 to 14.11
Time from test to death	17 eyes	$1.2 \pm 1.2$ yrs	16/17 <2 y, 11/17 <1 y

Type of field test: 12, program 30-2; 5, program 24-2. Algorithm: 16, standard Humphrey Field Analyzer 1; 1, full from prior strategy. Reliability: All 17 tests within reliability range: fixation loss and false-positive results.

matous eyes was divided by the mean density of normal eyes to give a percentage of normal value for points, clusters, hemifield, and whole retina. Sections were unsatisfactory for counting in 13 locations from seven eyes, representing missing data from 2.7% of the 476 possible locations in glaucoma-affected eyes.

Most retinal counting was performed by one observer, but some data were collected by a second observer. We performed comparisons of counting by these two persons, with masked, repeated counting of 10 sections from 10 eyes. The average difference for both intra- and interobserver counting was less than 2%, and analysis of variance found no significant difference either between or within persons ( $P > 0.5$  for both).

Some of the neuron-like cells that we included in our counting may have been amacrine cells. Our methodology required the higher resolution of resin embedding and 1- $\mu$ m sectioning, thus precluding the labeling of RGC bodies by immunohistologic techniques that are possible only in frozen or paraffin-embedded sections. In a previous investigation,<sup>4</sup> we determined the diameter of RGC bodies in wholemounts of human retina. This was not possible in enough of the present material, because of variation in tissue preservation.

In addition to counting RGC bodies, RGC axons in the optic nerve of both control and glaucomatous eyes were studied. The optic nerve was removed from each eye after razor slices were made for orientation, one slice at the superior nerve pole, and two slices nasally. A sample of each nerve 1-mm thick was embedded in epoxy resin, sectioned at 1  $\mu$ m and stained with toluidine blue. The number and size distribution of myelinated axons was determined using an image analysis system (Vidas; Carl Zeiss, Thornwood, NY).<sup>3</sup> Axon diameter was measured inside the myelin sheath. A trained observer edited each sampled area to remove capillaries, glial tissue, and degenerated axons that may have otherwise been included as intact nerve fibers. Optic nerve data were obtained on 16 eyes from 16 control persons, and from 13 eyes of 11 glaucoma patients.

There was a reasonable correlation between the number of RGCs estimated from counts in the retina compared with RGC axon number estimated from each eye (data for glaucomatous eyes: linear regression,  $R^2 = 0.35$ ,  $P = 0.022$ ,  $n = 13$  eyes of 11 persons; for one eye per person:  $R^2 = 0.35$ ,  $P = 0.05$ ,  $n = 11$ ). There may be differences between retinal and optic nerve counts, because the areas sampled in the retina represented locations only within the central 24°, whereas the optic nerve data were a 5% random sample of RGC axons from the entire retina. The number of RGC bodies counted was 1.4% of the estimated total number of RGCs in glaucomatous eyes and 1.0% in control eyes.

## RESULTS

Examination of data from normal eyes showed that RGC density was nearly 10 times higher in the central points nearest fixation than in more peripheral points (Fig. 1). These ranged from a low of  $16.2 \pm 5.7$  RGCs/mm retina at the nasal horizontal meridian point inferiorly to a high of  $153.8 \pm 51.9$  RGCs/mm at the point closest to the fovea, superiorly. There appeared to be no systematic difference between corresponding positions in the upper and lower retina.

The mean number of axons in the optic nerves of the control eyes was  $534,396 \pm 113,373$ . The average age for these persons without glaucoma was 76.4 years (Table 1). When total axon number was compared with age (Fig. 2), a significant decline in fibers was detected, with a slope indicating loss of 7205 fibers per year (linear regression:  $R^2 = 0.50$ ,  $P = 0.002$ ). This slope was used to calculate an age-normal relationship to estimate the percentage of normal axons present in each glaucomatous optic nerve. The variance of data at each individual data point for RGC counting was substantial, and meaningful regressions for age were not obtainable. Therefore, loss of RGC bodies was calculated by comparison to the average of all control eyes at the relevant location.

### RGC Data Compared with Mean Deviation in Visual Field Test

Among glaucomatous eyes, the percentage of normal RGC bodies for all 28 points in an eye was averaged to give a mean value for each eye. When a mean value for all glaucoma-affected eyes in this global average was calculated, the average percentage of normal in RGC number for all subjects with glaucoma ( $n = 17$ ) was 89.8% (for one eye per person: 82.2%;  $n = 13$ ), with the most damaged eye exhibiting 39.1% of normal RGCs. We compared the global indices from the visual fields to the global average percentage of normal RGC bodies (all points for each eye combined), and we compared the percentage of normal axon counts to the same field indices. For the absolute value of mean deviation (MD), the linear regression analysis found a modest correlation to RGC body percentage of normal ( $R^2 = 0.22$ ,  $P = 0.07$ ,  $n = 15$ ; Fig. 3; for one eye per person:  $R^2 = 0.32$ ,  $P = 0.05$ ,  $n = 12$ ). The slope of the regression suggested that a 0.05-dB loss in MD was associated with each 1% loss of RGCs. We hoped to compare RGC number to the probability value that the MD was within the normal Humphrey range, but most of the field values for this index were highly abnormal, precluding meaningful stratification. Nine of the 15 eyes had MD probability of 0.5%, four others were either 1% or 2%, and only one eye had values of 5%

TABLE 3. Glaucoma Historical Data

Known duration of glaucoma: mean $\pm$ 7.5 $\pm$ 10.4 y; range, 1–40 y	
Diagnoses	
Open-angle glaucoma or suspect	10 persons
Pigmentary glaucoma	1 person
Exfoliation glaucoma	1 person
Combined open/closed mechanism	1 person
Treated eye pressure level: 13/13 <25 mm Hg; 8/13 <21 mm Hg	
Cup-to-disc ratio (number eyes): 0.4 (3); 0.5 (3); 0.6 (2); 0.7 (1); 0.8 (2); 0.9 (2); no data (4)	
Refractive error: +2.00 to $-4.50$	
Surgery history: laser trabeculoplasty, 7/17 eyes; laser iridotomy, 2/17 eyes; lens implant, 3/17 eyes	

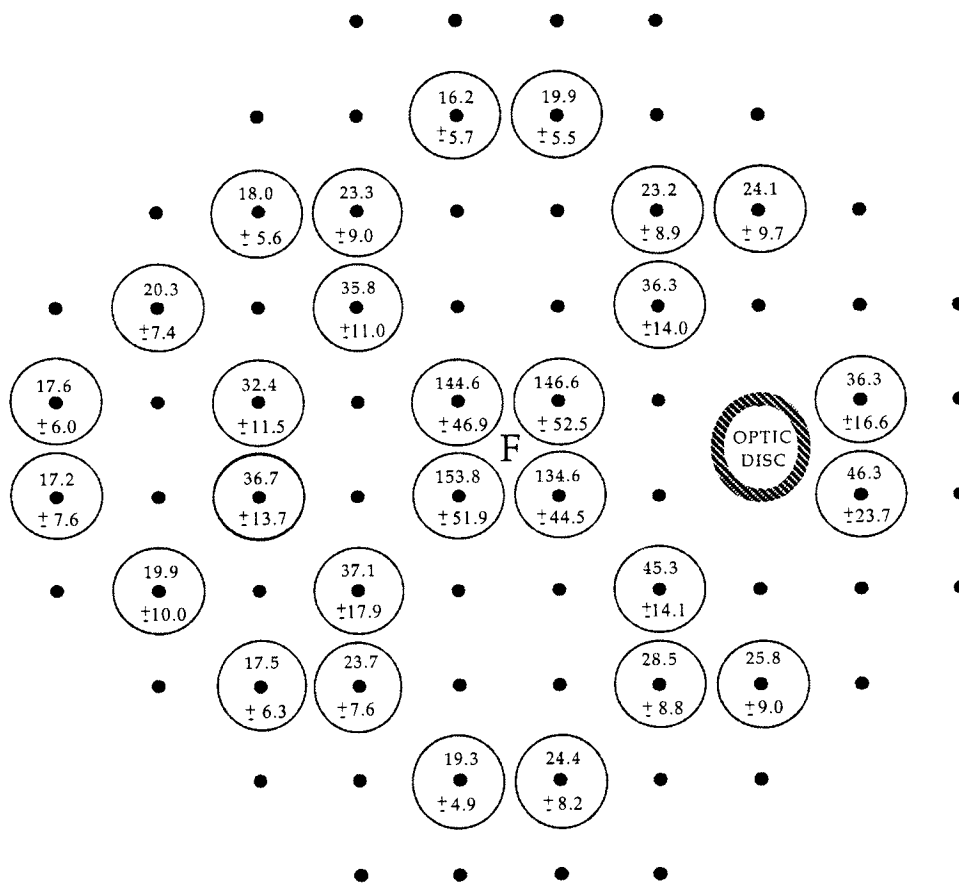


FIGURE 1. Positions of test points selected from the Humphrey 30-2 test (San Leandro, CA) in which RGC density estimates were obtained. Means ± SDs in normal retina of a right eye are given at each location measured in RGCs per millimeter. F, fovea.

or higher (one eye per person: six were 0.5%, four were 1% or 2%, and one was 5% or higher). There was also a modest correlation between the MD and the total axon number estimate in each eye (linear regression:  $R^2 = 0.43$ ,  $P = 0.15$ ,  $n = 12$ ; for one eye per person:  $R^2 = 0.27$ ,  $P = 0.13$ ,  $n = 10$ ).

**RGC Data Compared with Pattern SD in Visual Field Test**

The pattern SD (PSD) and corrected pattern SD (CPSD) indices were also compared with both the global average percentage of normal RGC bodies and the percentage of normal RGC

axons for the glaucomatous eyes. The correlation of RGCs remaining to PSD was of borderline significance (linear regression:  $R^2 = 0.17$ ,  $P = 0.13$ ,  $n = 15$ ; for one eye per person:  $R^2 =$

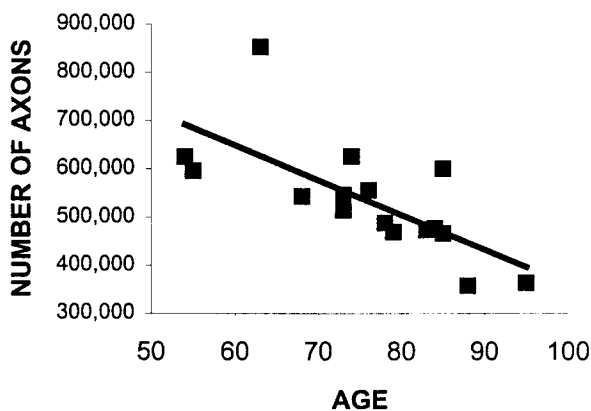


FIGURE 2. The estimated number of optic nerve axons in normal eyes decreases with older donor age, with a slope of 7205 axons lost per year (linear regression:  $R^2 = 0.50$ ,  $P = 0.002$ ).

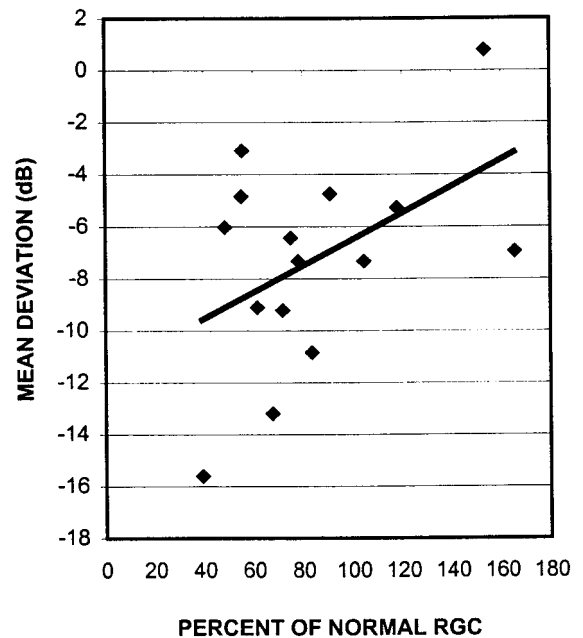


FIGURE 3. The MD index value in Humphrey visual field (San Leandro, CA) was more abnormal (– MDs) in eyes with fewer RGCs, measured as a percentage of normal cell counts (linear regression:  $R^2 = 0.32$ ,  $P = 0.05$ ).

= 0.15,  $P = 0.21$ ,  $n = 12$ ). Total RGC axons remaining (percentage of normal) and PSD absolute value in decibels were more closely correlated (Fig. 4; exponential regression:  $R^2 = 0.55$ ,  $P = 0.0056$ ,  $n = 12$ ; for one eye per person:  $R^2 = 0.52$ ,  $P = 0.006$ ,  $n = 10$ ). The CPSD absolute values had a similarly low correlation to RGC body or axon estimates. The probability that PSD or CPSD fall within the Humphrey normal range (instead of their absolute values) had a stronger relationship to RGC loss. For CPSD probability grading of either normal or 10%, mean percentage of normal RGCs was 96.9% ( $n = 4$  eyes; for one eye per person: 73.9%,  $n = 3$  eyes), whereas for those with probabilities of 5% or lower, the mean was 72.7%,  $n = 10$  eyes (for one eye per person: 72.1%,  $n = 8$  eyes). A subset of the latter group, those with probability of 0.5% had a mean of 63.3% ( $n = 4$  eyes; for one eye per person: 60.5%,  $n = 3$  eyes).

**RGC and Field Data in the Superior Compared with the Inferior Hemiretina**

Data from RGC body counts in the inferior and superior retina were correlated to the average loss of sensitivity in each hemifield (decibels) and percentage of normal RGC values averaged at the 14 points in the corresponding, opposite hemifield. Again, regression modeling showed modest relationships, with the inferior field (superior retina) data shown in Figure 5 for all 17 eyes ( $R^2 = 0.31$ ,  $P = 0.02$ ,  $n = 17$ ; for analysis with only one eye per person:  $R^2 = 0.32$ ,  $P = 0.05$ ,  $n = 13$ ). The slope of the regression for all eyes indicates loss of 0.084 dB per 1% RGC loss.

One method to minimize variability in both field and retinal data would be to compare upper to lower retina in the same eye. Although retinal preservation and field test reproducibility probably vary more among persons than between the two hemifields of the same eye. In 11 eyes of nine glaucomatous persons, we calculated the difference between the

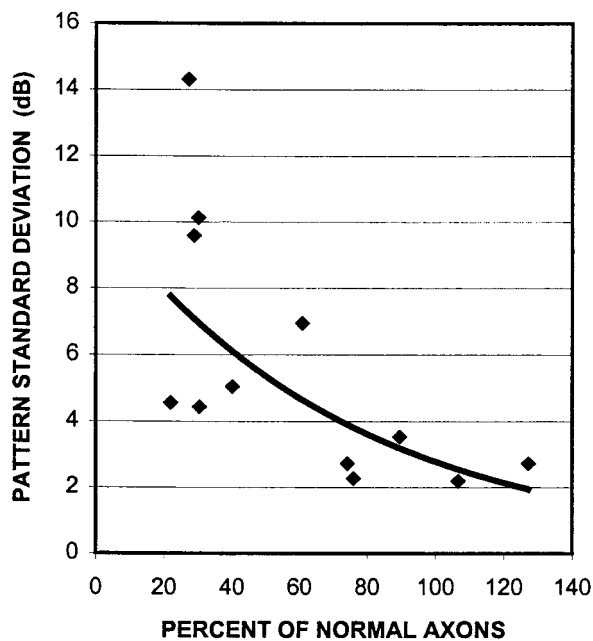


FIGURE 4. Abnormally higher values of PSD index in the visual fields was associated with greater loss of optic nerve axons (percentage of normal; exponential model,  $R^2 = 0.55$ ,  $P = 0.0056$ ).

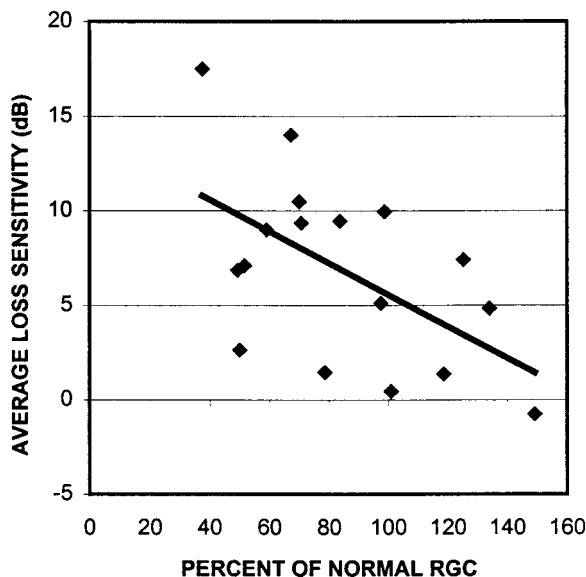


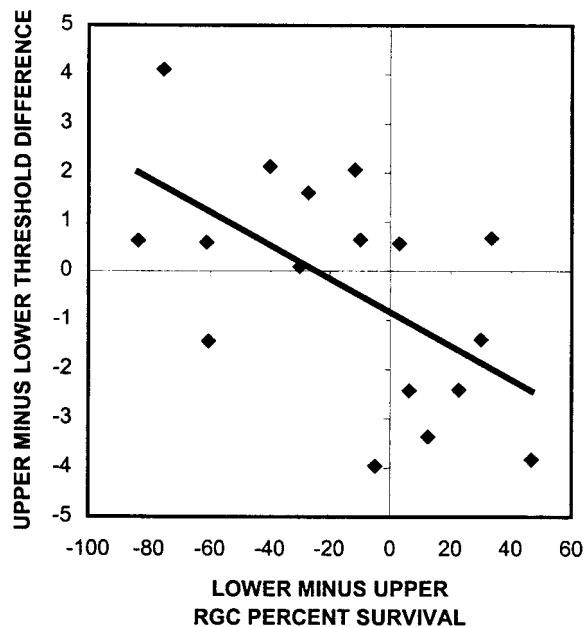
FIGURE 5. The average loss of sensitivity (in decibels) of all points in the lower visual field was correlated with the percentage of normal RGCs in the corresponding upper retina. The intercept for the regression line indicated that complete RGC loss would result in a 14-dB loss (linear regression:  $R^2 = 0.31$ ,  $P = 0.02$ ).

average lower and upper hemifields in percentage of normal RGCs, as well as the corresponding upper minus lower loss of sensitivity in the field. When upper retina RGC loss was greater, lower field damage was greater (and vice versa). The regression relation was highly significant ( $R^2 = 0.51$ ,  $P = 0.013$ ,  $n = 11$ ; for one eye per person:  $R^2 = 0.39$ ,  $P = 0.07$ ,  $n = 9$ ), and the slope indicated a 2-dB loss for each 10% RGC loss (an approximately 5-dB loss for 25% cell damage).

**Cluster and Point-by-Point Comparison of RGC and Field Data**

Our method included two or three points from each of the five clusters in the Glaucoma Hemifield Test of the Humphrey perimeter. As with the upper-lower retina comparison, the data comparing cluster 1 (Fig. 6; nearest the fovea) found a highly significant relation between the difference in two upper points and two lower points in percentage of normal RGCs compared with the difference in their threshold loss in the corresponding test points (Fig. 7;  $R^2 = 0.34$ ,  $P = 0.014$ ,  $n = 17$ ; for one eye per person:  $R^2 = 0.28$ ,  $P = 0.07$ ,  $n = 13$ ). The other clusters had insignificant relationships between histologic and functional findings. Cluster 1 has the highest density of RGCs in the retina, whereas the other clusters have substantially lower density. These low densities provide few RGCs and wide variability in attempting to make these correlations at clusters other than cluster 1.

When each of the 420-individual-point RGC body data were compared with their corresponding threshold loss, a significant relationship ( $P < 0.001$ ) was shown, but the regression explained only a tiny fraction of the variability seen in sensitivity values ( $R^2 = 0.03$ ; analysis using only one eye per person:  $R^2 = 0.05$ ,  $P < 0.001$ ). However, the data for the total deviation probability for each point compared with the percentage of normal RGC body values of each were more illuminating (Table 4; all eyes included). There was a clear loss of



**FIGURE 6.** Comparison of data for two points in the upper visual field that fall within cluster 1 of the Glaucoma Hemifield Test (Humphrey, San Leandro, CA) and their two mirror-image locations in the inferior visual field. Cluster 1 consists of points close to central fixation. The average percentage of normal RGCs in the two upper retinal locations was subtracted from that of the paired lower locations (*horizontal axis*). The loss from age-normal values in visual field threshold (in decibels) for the lower visual field pair of points was subtracted from that of the upper field pair (*vertical axis*). The data points are grouped, as expected, in either the *upper left* or *lower right* quadrants (linear regression:  $R^2 = 0.34$ ,  $P = 0.014$ ).

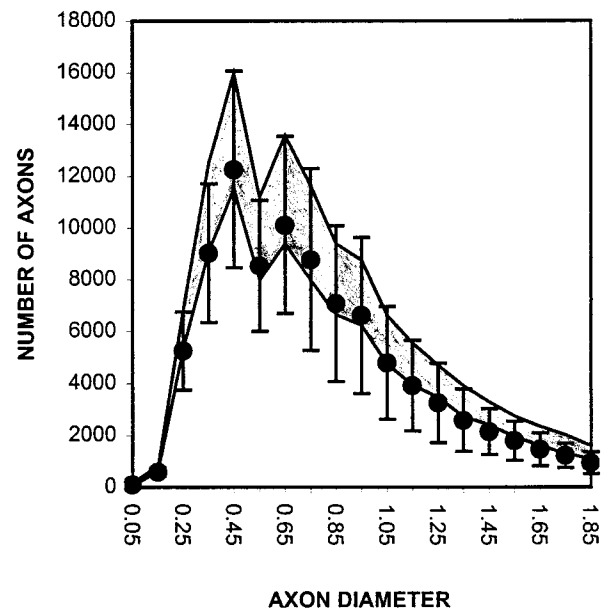
RGCs for points with probabilities of 2% or less. At the 0.5% level, the data suggest an average RGC loss of 29%.

### Comparison of RGC Axon Diameter in Glaucomatous and Control Eyes

The diameter of axons in the optic nerve was measured by the image analysis system for 11 eyes of 11 glaucomatous patients and 16 eyes of 16 normal subjects. The normal and glaucomatous fiber diameter distributions are shown in Figure 7. The normal distribution is skewed toward larger fibers and exhibits two peaks at internal axon diameters less than  $1 \mu\text{m}$ . The mean values for glaucoma data groups fall within 1 SD of the normal fiber distribution among the small fiber groups but outside this limit for larger fibers. The glaucoma axon distribution shows no tendency to increased fiber diameter among the smaller diameter bins, compared with the normal data, indicating no evidence for shrinkage of a substantial number of fibers. The proportion of axons remaining at each diameter in the glaucoma patients showed a significantly greater preservation of smaller fibers compared with larger fibers (Fig. 8; linear regression:  $R^2 = 0.78$ ,  $P < 0.001$ ).

## DISCUSSION

The findings of this extensive evaluation of RGC bodies in the retina and their axons in the optic nerve provide more precise estimates for the relationship between RGC loss and visual field



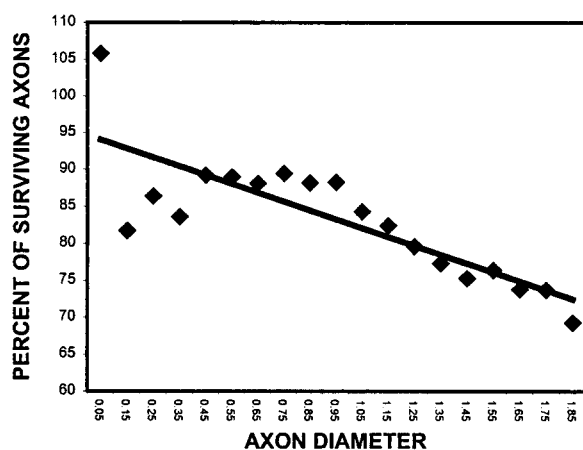
**FIGURE 7.** Optic nerve axon diameter data from 16 eyes of 16 normal and 11 eyes of 11 glaucoma subjects. The normal axon distribution (mean  $\pm$  1 SD, *shaded region*) shows two peaks in the diameter range less than  $1 \mu\text{m}$  and a skew toward larger values. The axon distribution of the glaucomatous eyes (*circles*, with error bars) falls generally within 1 SD of the normal mean for small fiber diameters and at or below this level for larger diameters.

abnormality than previously reported. To our knowledge, before this study, RGC counting had been compared with automated field tests in only five eyes.<sup>4,5</sup> Two of these eyes<sup>5</sup> had normal fields and had no decrease in RGCs, whereas in the other three eyes, there appeared to be loss of a substantial minority of RGCs before detection of loss of sensitivity at the 5-dB level. The data presented here consist of eyes with a mean of 89.9% normal RGC body number. This collection of eyes with relatively early damage may optimize the chance to correlate mild loss in RGCs with initial field loss. The data confirm the loss of many RGCs before detection, whether field abnormality is judged by global measures, comparisons of upper to lower field test results, or individual test point probability of abnormality. In comparisons of CPSD probability, upper-lower retinal decibel loss, or individual point probability, 25% to 35% of RGCs were dead in fields that satisfied typical clinical criteria for abnormality.

The density of RGC bodies was 10 times higher in the perifoveal retina than for retinal locations corresponding to points at the outer measurement zone of the field test ( $20$ – $30^\circ$  from fixation). If we assume that some of the cells we counted

**TABLE 4.** Comparison of Stratified Total Deviation Probability for Individual Points Compared with Percentage Normal RGC Data

Total Deviation Probability	Mean % Normal RGCs	Number of Points
Normal	90.1	175
5%	96.7	58
2%	89.6	33
1%	76.8	45
0.5%	71.5	97



**FIGURE 8.** The glaucoma-affected eyes (triangles, regression line) are represented as a percentage of normal axons surviving at each diameter. Note that nearly 90% of the number of small fibers are present, whereas only 70% of fibers among the larger groups remain (linear regression:  $R^2 = 0.78$ ,  $P < 0.001$ ).

in the ganglion cell layer were actually amacrine cells and not RGCs, our estimates of percentage of loss would actually be understated. For example, if 50% of identified neurons were amacrine (a very unlikely result in the central retina), then our estimate of 50% loss would have had to involve loss of 100% of the original RGCs, with the 50% non-RGCs remaining. In addition, if amacrine cells are included in our counts and if they atrophy in proportion to the loss of RGCs, then our estimates would be unaffected.

Some measures of field test results could not be closely correlated with the RGC numbers. There is substantial variation in the total number of RGCs from one eye to another, placing relatively broad confidence limits on any estimate of histologic damage. Furthermore, there is very significant variability in field test results both among persons and for the same subject within and between tests. The reproducibility of our histologic counting methods is excellent and adds only minimally to the variability in correlations. Finally, some field measures would not be expected to correlate with glaucoma damage very closely, because they are measures of general sensitivity loss that can be influenced by other disorders, age, and test conditions. For example, the MD index was less specifically linked to glaucoma damage than CPSD.<sup>17</sup>

The validity of our RGC counting was supported by the correlation of the retinal data with the optic nerve fiber counting in the same eyes. The number of fibers in the normal optic nerve has varied from 600,000 to 1.2 million in various reports.<sup>18-24</sup> Many investigators have either suggested or statistically demonstrated that RGC numbers decline with age. Age-related loss estimates have varied from as few as 2,000 to as many as 12,000 RGCs dying per year. The normal number of RGCs in this report was lower, and our estimated loss with age was higher than our own previous estimates. This probably resulted from the substantially older age of our control subjects (compared with past reports), who were selected to match the age of the glaucoma patients. Inspection of data from other investigators reveals that the loss of RGCs with age may accelerate after middle age.<sup>24</sup> The control subjects of this study were nearly 20 years older than those in our previous reports,<sup>3,23</sup> in which only a modest loss of RGCs was estimated

with aging. If we assume that the loss of RGCs increases with advancing age, subjects with glaucoma who have loss of the majority of RGCs could undergo progressive impairment with an age-related loss of 7000 fibers per year, despite any effort to treat the disease.

We have shown that larger RGCs are preferentially susceptible to death from glaucoma in human eyes,<sup>3,4,6,8,9,11,12</sup> and this was corroborated in studies of the retina,<sup>5</sup> the optic nerve,<sup>7</sup> or the lateral geniculate body<sup>10</sup> of persons with glaucoma and in experimental monkeys.<sup>6,12</sup> Selectivity is not always demonstrable in the monkey model,<sup>13,14,27</sup> especially when very rapid damage is caused by short-term, high intraocular pressure. If RGC axons were to decrease their diameter before death, an apparently selective loss of larger axons might be simulated. We have previously demonstrated that our data are not compatible with this hypothesis.<sup>8</sup> The present glaucoma axon data show no shift of axon diameter to smaller sizes. Axon diameter and cell body size are correlated with functional RGC behavior, and psychophysical tests that exploit the loss of the functions subserved by larger RGCs including scotopic,<sup>28</sup> motion,<sup>29</sup> and frequency-doubling paradigms<sup>30</sup> show promise in glaucoma diagnosis. The translation of anatomic selectivity into useful psychophysical tests depends on the sensitivity with which loss of particular RGCs can be detected by functional testing.<sup>15,31</sup>

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