

Ganglion Cell Losses Underlying Visual Field Defects from Experimental Glaucoma

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PURPOSE. To investigate the relationship between ganglion cell losses and visual field defects caused by glaucoma.

METHODS. Behavioral perimetry and histology data were obtained from 10 rhesus monkeys with unilateral experimental glaucoma that was induced by argon laser treatments to their trabecular meshwork. After significant visual field defects had developed, the retinas were collected for histologic analysis. The ganglion cells were counted by light microscopy in cresyl violet-stained retina sections, and the percentage of ganglion cell loss (treated to control eye counts) was compared with the depth of visual field defect (treated to control eye thresholds) at corresponding retinal and perimetry test locations. Sensitivity losses as a function of ganglion cell losses were analyzed for Goldmann III, white and Goldmann V, and short- and long-wavelength perimetry test stimuli.

RESULTS. The relationship between the proportional losses of ganglion cells and visual sensitivity, measured with either white or colored stimuli, was nonlinear. With white stimuli, the visual sensitivity losses were relatively constant (approximately 6 dB) for ganglion cell losses of less than 30% to 50%, and then with greater amounts of cell loss the visual defects were more systematically related to ganglion cell loss (approximately 0.42 dB/percent cell loss). The forms of the neural-sensitivity relationships for visual defects measured with short- or long-wavelength perimetry stimuli were similar when the visual thresholds were normalized to compensate for differences in expected normal thresholds for white and colored perimetry stimuli.

CONCLUSIONS. Current perimetry regimens with either white or monochromatic stimuli do not provide a useful estimate of ganglion cell loss until a substantial proportion have died. The variance in ganglion cell loss is large for mild defects that would be diagnostic of early glaucoma and for visual field locations near the fovea where sensitivity losses occur relatively late in the disease process. The neural-sensitivity relationships were essentially identical for both white and monochromatic test stimuli, and it therefore seems unlikely that the higher sensitivity for detecting glaucoma with monochromatic stimuli is based on the size-dependent susceptibility of ganglion cells to injury from glaucoma. (*Invest Ophthalmol Vis Sci.* 1999;40:2242-2250)

Static threshold perimetry has become the standard procedure for the assessment of the ocular effects of glaucoma.¹⁻⁵ The diagnosis of a patient's clinical status based on visual fields requires both statistical and mechanistic evaluation of the data. First, the individual's data are automatically compared with age-matched normal data to determine

whether their test results are significantly different from expected values.^{6,7} The statistical analyses, which are presented as probability plots, global indices,⁷⁻¹¹ and glaucoma hemifield tests,^{12,13} are well-developed procedures for the identification of clinically significant defects that do not require assumptions about the mechanism or cause of a visual field abnormality.

In contrast, the mechanistic interpretation of the visual fields of a glaucoma patient is directly based on a fundamental hypothesis for the relationship between visual sensitivity and retinal ganglion cells. The simplest form of the neural-sensitivity hypothesis is that the proportion of normal retinal ganglion cells determines perimetry thresholds.¹⁴⁻¹⁷ Thus, for an eye with glaucoma, it is assumed that a reduction in visual sensitivity is a result of the loss of retinal ganglion cells and that retinal areas with greater losses of sensitivity have undergone greater glaucomatous losses of ganglion cells.

The relationship between the death of ganglion cells and reduction of visual field sensitivity is important because it provides a method for quantifying the ocular effects of glaucoma by psychophysical testing. However, despite its importance, only a single empiric investigation has been reported.¹⁸ In the analysis of the results of that study, which have been

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very influential on subsequent research on early detection and diagnosis of glaucoma, it was proposed that the proportional decreases in visual sensitivity and ganglion cell losses are linear, with a slope of 0.2 to 0.4 dB loss/percent loss of ganglion cells. The linear regression analysis also implied that there is a threshold ganglion cell loss for clinically significant visual losses, with a 20% to 50% loss of ganglion cells required to produce a 6 dB loss by static perimetry. However, the determination of a precise neural-sensitivity relationship was complicated by substantial variations that were found for the sensitivity losses associated with any given amount of ganglion cell loss.

Because of its significance for understanding the ocular effects of glaucoma, we endeavored to extend the study of retinal ganglion cell losses that underlie visual field defects via the use of experimental glaucoma in macaque monkeys.¹⁹⁻²² An animal model for these investigations would provide several advantages, primarily technical, that should allow better tests of the relationship between ganglion cell and visual losses. For example, the data from monkeys may be less variable than data from donated human eyes because experimental glaucoma is produced unilaterally, which allows sensitivity versus neural losses to be assessed by differences between the treated and control eyes of a single subject. A further advantage for these studies is that retinal tissue can be collected from monkeys at various stages of glaucoma to determine the neurologic damage underlying the range of mild-to-severe visual defects. In addition, with experimental glaucoma, the retinal tissue is fixed and processed immediately after the death of the animal to provide excellent material for histologic analysis.

The relationship between ganglion cell losses and visual field defects from glaucoma in monkeys and human patients should be comparable because the physiological and psychophysical effects of glaucoma are similar in the two species. With respect to pathophysiology, several investigations have demonstrated close agreement in the optic neuropathies caused by the natural glaucoma of patients and from experimentally elevated intraocular pressure in monkeys.²³⁻²⁶ Likewise, behavioral measurements taken with computerized threshold perimetry have demonstrated analogous results for monkeys and humans for both normal visual fields²⁷ and progressive glaucomatous visual field defects.^{22,28} However, in both cases there is a question of the most appropriate psychophysical test for the evaluation of ocular defects because visual field defects occur earlier and appear to be more advanced with monochromatic than white perimetry test stimuli.²⁹⁻³⁴ It is, therefore, speculative as to whether perimetry with stimuli that might bias detection to certain subpopulations of ganglion cells could provide a more accurate assessment of the neural damage caused by glaucoma.³⁵⁻³⁷

The purpose of the present study was to investigate these basic issues of the neural-sensitivity relationship that provides the foundation for interpretation of visual fields in clinical diagnosis and the assessment of progression of glaucomatous neural damage. The investigation was designed to correlate psychophysical and histologic measurements of the ocular effects of glaucoma on a point-by-point basis. These data were used to derive a quantitative relationship between the reduction in visual sensitivity and the loss of ganglion cells for subjects showing various grades of visual field degeneration. Neural-sensitivity relationships were determined for two classes of perimetry stimuli: Goldmann III, white stimuli, for

conventional clinical perimetry,^{1,2} and Goldmann V, monochromatic stimuli of either 460 nm, for short-wavelength perimetry,^{28,35,38} or 620 nm, for opponent-mechanism perimetry.^{28,35,38}

METHODS

Subjects

The subjects for the investigations were 10 of the adult male rhesus monkeys (*Macaca mulatta*) that had been subjects in a prior study of intraocular pressure and visual field defects.²² The intraocular pressure of each monkey's right eye was elevated by Argon laser treatment of the trabecular meshwork.¹⁹⁻²¹ Details of the laser procedure and intraocular pressure measurements have been published previously.²² The untreated left eyes served as controls. All of the experimental and animal care procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Perimetry

The methods and procedures for behavioral perimetry have been reported previously.^{27,38} In brief, the visual fields of the monkeys were measured with a Humphrey Visual Field Analyzer (model 630; Humphrey Allergan, San Leandro, CA). Several superficial modifications were made to accommodate monkeys as subjects and to provide additional behavioral control, but these modifications did not alter the standard testing protocol or data analysis programs. During testing, the monkeys were seated in a custom-made primate chair that provided adjustments for alignment of their eyes at the correct viewing location and placement of their mouths on the juice spout used to deliver behavioral rewards. While in the chair, they were able to grasp the response lever that was used for their behavioral responses during visual field testing. Their thresholds for the fixation and perimetry stimuli were obtained by a psychophysical method, a criterion response-time paradigm for monkeys, which retains the essential requirements of the test procedure used for patients. The principal components of the procedure for the monkey subjects were as follows: The monkeys were trained to press and hold down their response lever to initiate a trial and, subsequently, to release the lever in the presence of a visual stimulus. Because the time of the occurrence of the stimulus was varied randomly, if the monkey's lever release was closely correlated to the visual stimulus presentation (within a 900-msec response interval), then the response was operationally defined as a true-positive response (a hit), and it was rewarded. Alternatively, if the monkey's lever release was beyond the response interval, it was considered as a false-negative response (a miss), and it was neither rewarded nor punished. The test stimulus in any trial could be the central fixation stimulus or one of the peripheral perimetry stimuli. The locations and intensities of the peripheral test stimuli were determined by the perimeter's internal software.

The monkeys' visual fields were measured each week using the Humphrey Field Analyzer's C24-2 full-threshold program. Visual fields data were collected on consecutive days with three forms of perimetry stimuli: the clinical standard Goldmann III white test stimuli and two narrow-band monochromatic stimuli using Goldmann V targets. In contrast to the white stimulus, the specific wavelengths of the colored stimuli isolated specific, independent detection-mechanisms at all lo-

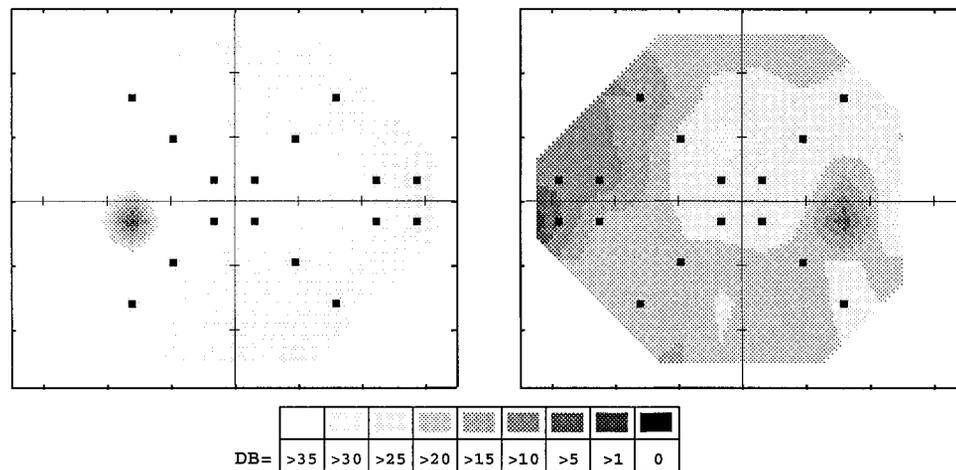


FIGURE 1. Examples of visual field data for the treated (*right panel*) and control (*left panel*) eyes of a monkey with experimental glaucoma (subject OHT-23, see Ref. 22 for details of the laser treatment). Data for the grayscale plots were obtained from the 24-2 full-threshold program of the Humphrey Field Analyzer with a Goldmann III white stimulus (see text for details). The *filled squares*, which are superimposed on the visual field plots, designate the test-field locations for retinal tissue samples collected to quantify sensitivity-neural relationships for visual field defects caused by glaucoma.

cations in the visual field,³⁸ even in monkeys with visual field defects of more than 20 dB.²⁸ For these tests, a 460-nm stimulus was used to assess visual field sensitivities for the short-wavelength mechanism, and a 620-nm stimulus was used for opponent-mechanism perimetry.

The sequence of perimetry measurements for each monkey was continued until reliable visual field defects were obtained with the white stimuli, and then the retinas were collected for histologic analysis. An example of the extent of visual field defects at the termination of behavioral perimetry is presented in Figure 1. The perimetry data for this monkey demonstrate a form of visual field defects that also has been reported for patients with prolonged high intraocular pressure.¹⁸ In this subject, there was a generalized reduction in sensitivity of the treated eye (right plot), with respect to the control eye (left plot), although the losses of sensitivity were somewhat more pronounced in the superior nasal field. These effects were also revealed by the perimetry global indices, with a mean deviation (MD) of -8.54 dB ($P < 0.02\%$) and a corrected pattern SD of 3.96 dB ($P < 10\%$). Across all of the subjects, the behavioral experiments were terminated for some monkeys with mild mean deviation defects and for others with more advanced glaucomatous losses. The point-wise sensitivity losses across the visual field were taken as the difference, in decibels, between the control and treated eyes at equivalent locations in the visual fields.

Histologic Analysis

Within a few days after the final visual field test, the monkeys were deeply anesthetized and their eyes were enucleated. The retinal tissue processing for the histologic analysis has been described previously.^{39,40} In essence, the posterior segments of the eyes were fixed by an immersion overnight in 2% paraformaldehyde and 2% glutaraldehyde at 4°C. The eyes were then transferred and stored at 4°C in phosphate buffered 4% paraformaldehyde (pH 7.3).

Tissue samples for 16 specific perimetry test sites, illustrated in Figure 1, were taken from comparable retinal locations in the control and treated eyes. For some monkeys, retinal samples from all the locations were analyzed; however, to provide retinal tissue for other studies,³⁹ only selected samples were taken from some of the monkeys (a total of 95 samples from the 10 monkeys). The retinal locations for tissue samples were determined by the usual conversion ratio of 1 mm retina per 4° of visual angle.⁴¹ This ratio was also verified for the present study by comparing the distance from fixation to the center of the perimetrically plotted blind spot and the direct measurement of the distance from the fovea to the center of the optic nerve head.⁴⁰

In preparation for counting the ganglion cells, the retinal tissue samples were embedded (LR White resin; Ted Pella, Redding, CA) and, subsequently, sectioned (thickness, 1 μm ; radial sections) and stained (0.5% cresyl violet). Examples of the histology preparations are presented in Figure 2. The examples demonstrate that the morphology of the retinas of treated and control eyes of the monkeys was essentially identical, except for the reduced number of cells in the ganglion cell layer.⁴² The amount of ganglion cell loss was quantified by counting the neurons under light microscopy with 100 \times magnification. All the neurons were counted in a 1- μm segment from each of 10 sections separated by a minimum of 10 μm . The cell densities of the ganglion cell layers were calculated using Abercrombie's method⁴³ for deriving densities from sectioned tissue. Displaced amacrine cells were not excluded from the counts because it was demonstrated previously that the density of these cells appears unaffected even in eyes with long-standing high intraocular pressure.⁴² Therefore, any difference in cell density between treated and control eyes largely will be a reflection of a loss of ganglion cells, quantified by the percent difference of the treated eye compared with the control eye. However, because displaced amacrine cells

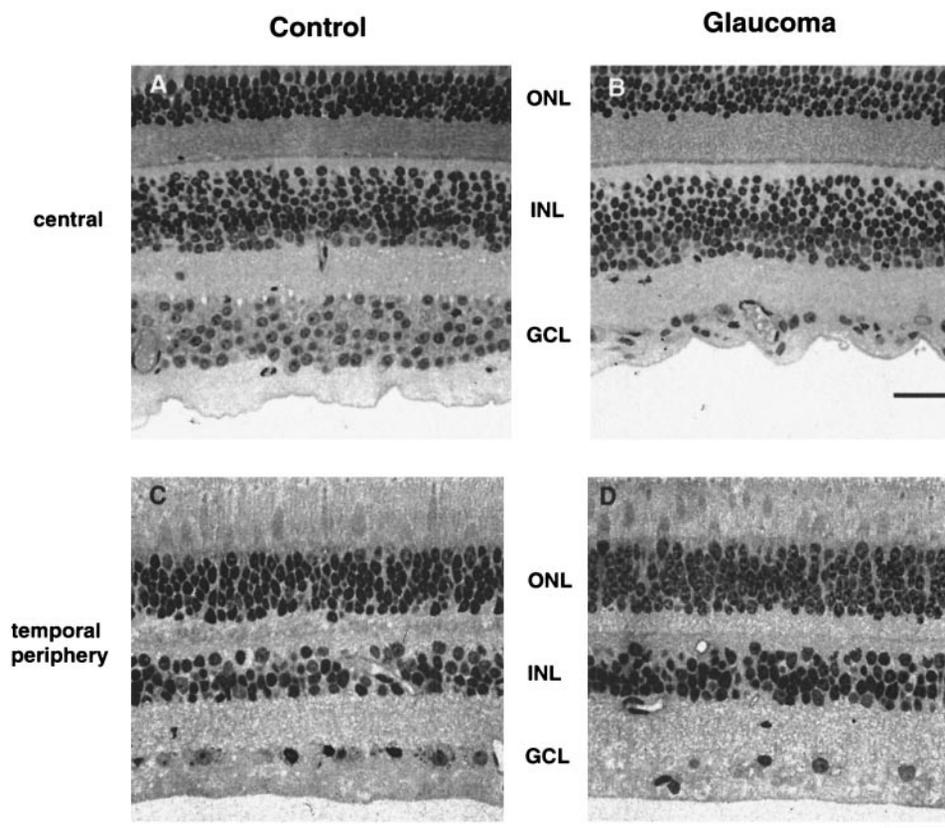


FIGURE 2. Cresyl violet-stained sections from control and glaucomatous eyes of monkeys OHT-11 (A and B) and OHT-8 (C and D). The ganglion cell layer (GCL) of the central retinal of the glaucomatous eye of OHT-11 shows a 97% cell loss (*cf.*, A and B). The temporal peripheral retina (superior-nasal visual field) of OHT-8 shows a 75% retinal ganglion cell loss (*cf.*, C and D). There were no apparent alterations of the inner nuclear layers (INL) or outer nuclear layers (ONL) of either monkey.

range from approximately 3% in the central retina to 25% in the temporal mid-peripheral retina,⁴¹ the absolute percentage of ganglion cell loss is slightly higher than reported.

RESULTS

White Test Stimuli

Because in the standard clinical protocol Goldmann III white test stimuli are used for visual field measurements, it is most important to determine the neural versus sensitivity relationship for this conventional measurement. These results are presented in Figure 3. Figure 3A shows the point-by-point relationships for perimetry sensitivity losses as a function of the percentage reduction in retinal ganglion cells caused by experimental glaucoma. Although the neural versus sensitivity data, which cover the full extent of possible ganglion cell losses (2%–99%) and perimetry sensitivity losses (2–36 dB), have considerable variability, there is an important trend of clinical significance. For example, there was an essential dichotomy in the magnitude of ganglion cell losses underlying sensitivity losses of less than or greater than 15 dB. As illustrated by the dashed lines on the graph (Fig. 3A), sensitivity losses that were greater than 15 dB were almost always associated with ganglion cell losses of more than 70%. For less severe visual field defects, the vast majority of sensitivity losses less than 15 dB are caused by ganglion cell losses of less than 70%.

An adapted plot that involved averaging within fixed abscissa ranges (Fig. 3B) illustrates the quantitative relationship between sensitivity and neural losses. To construct the plot, ganglion cell losses were grouped into 7 bins, each

containing at least a dozen samples, and the mean and variance for the sensitivity losses were derived for each bin. The result of creating this form of presentation demonstrates that the data are best fit by a power function with 2 variables. One variable is the exponent and determines the shape of the function over the accelerating portion of the curve, whereas the other variable sets the overall vertical position. The vertical placement of the function is interesting because it suggests that on average there can be approximately 6 dB loss in sensitivity before measurable ganglion cell loss occurs. However, in a more practical sense, the analysis shows that sensitivity losses that are not well correlated with ganglion cell losses of less than approximately 50%, and then with greater cell losses the relationship is relatively linear (0.42 dB/percent cell loss).

The population data in Figure 3 demonstrate the form and variability of the sensitivity-neural relationship across subjects, but it is also important to examine the results from individual subjects. The individual subjects' data may establish whether the variability seen in the group data is a result, primarily, of the effects of experimental glaucoma across subjects or within single subjects. The relevance of the question arises because if the major source of variability is intersubject differences in the effects of glaucoma then the depth of perimetric defects could be a reliable assessment of the progression of neural defects for a given subject.

The individual relationships between sensitivity losses and neural losses are illustrated in Figure 4, which are data from two representative monkeys, one with advanced visual defects and the other with more moderate visual field defects. For each animal, the visual fields are presented in the left panels (Figs.

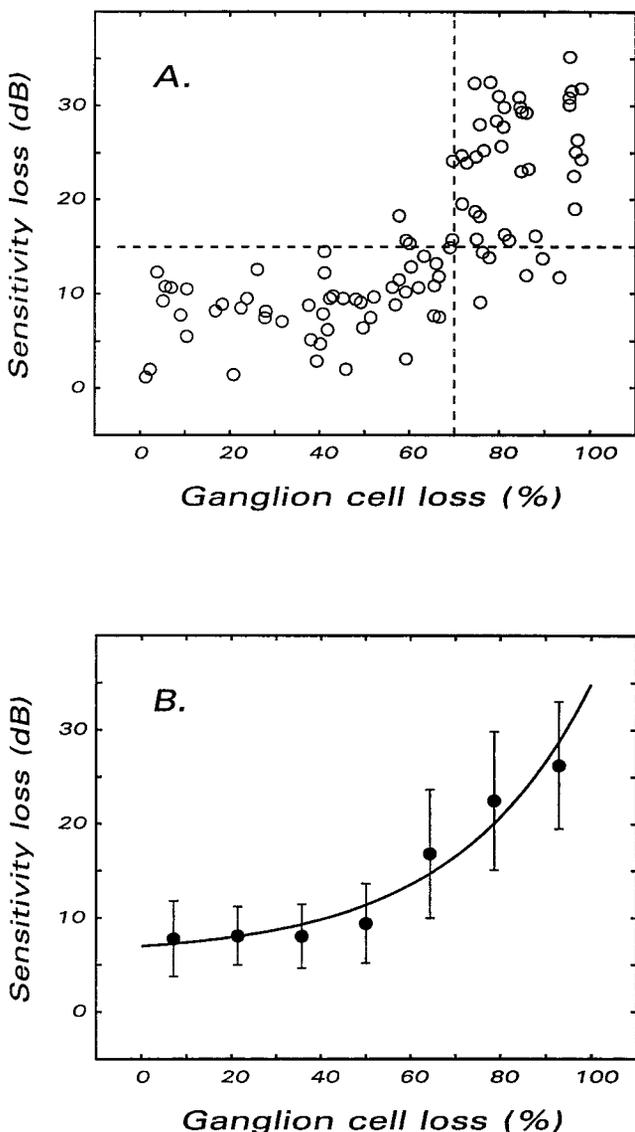


FIGURE 3. The loss of visual sensitivity (i.e., control eye - experimental eye perimetry thresholds for Goldmann III, white stimuli) as a function of the loss of ganglion cells (i.e., percent loss for experimental eye compared with the control eye) caused by experimental glaucoma in macaque monkeys. (A) 95 samples of the sensitivity-neural relationship from the 10 monkeys. *Dashed lines* are superimposed on the data to illustrate that visual field defects of >15 dB are almost always caused by ganglion cell losses $>70\%$. (B) adapted plot illustrates the quantitative relationship between sensitivity and neural losses. The data represent the mean \pm SD value for the sensitivity losses caused by ganglion cell losses in each of 7 equal-sized cell-loss ranges. The fitted curve is a power function, $S = bt + 10^{[(G-st)/100]}$, where S = sensitivity loss (in decibels), G = ganglion cell loss (as a percentage), st = shape parameter for the rapid acceleration portion of the curve, and bt = a position parameter for the vertical position of the function.

4A, 4C) and sensitivity versus ganglion cell losses in the right panels (Figs. 4B, 4D). The curves superimposed on the sensitivity-cell loss graphs represent the power function fitted to the group data (Fig. 3A).

By inspection, the relationship for the individual data from subject OHT-9 (Fig. 4A) appears to be tighter than for the group data, and the increases in the visual field defects caused

by ganglion cell losses are systematic. However, it should be noted that the data for this monkey are in the area of highest correlation for the group data. The ganglion cell loss was greater than 50% at every sample location, and the mean deviation of the field data was -16.77 dB. Another interesting point is that the three data points that represent the largest discrepancies, falling in the lower-right area of the graph, are from sample locations corresponding to $3 \times 3^\circ$ eccentricities at both test locations in the superior hemifield and the $3 \times 3^\circ$ location in the inferior nasal quadrant. It was not unusual, in fact, that the most extreme outlying points were those nearest the fovea.

Subject OHT-8 (Figs. 4C, 4D) exhibited less severe perimetry defects (MD = -8.94 dB). As for subject OHT-9, the function for the group data seems to be a satisfactory fit for the individual's data. OHT-8's data are also similar to the larger data set in the range from nearly 0% to 70% loss of ganglion cells, where the loss of visual sensitivity was typically less than 15 dB. Thus, the data from this subject provide substantiating evidence of the low correlation between sensitivity deficits and ganglion cell losses when ganglion cell losses are less than 50% to 70%.

Monochromatic Test Stimuli

The neural versus sensitivity relationship with short- and long-wavelength monochromatic test stimuli was investigated because narrow-band-colored stimuli have been advocated as a technique for restricting visual detection thresholds to specific subpopulations of ganglion cells and, thereby, producing diagnostic visual field defects at an earlier stage of glaucoma than with white stimuli.^{30,31} Thus, it could be that monochromatic stimuli provide a more accurate assessment of early ganglion cell losses.

The results of these investigations (Fig. 5) are presented in the data-reduction format that was used with white stimuli in Figure 2B. However, for these data, to compensate for the smaller range of normal thresholds with colored stimuli, the range of ordinate values was rescaled to cover a reduced range of sensitivity losses. Figure 5A illustrates the neural versus sensitivity relationship for a 460-nm test stimulus, which is a stimulus that produces perimetry thresholds mediated by the short-wavelength detection mechanism,²⁸ comparable to SWAP (short-wavelength automated perimetry methods).³³ The perimetry data shown in Figure 5B were obtained with opponent-mechanism perimetry by using a 620-nm test stimulus.²⁸

With the thresholds normalized, the neural versus sensitivity relationship for perimetry with either of the monochromatic stimuli appears to be very similar to that for white stimuli. Although, the values for the descriptive variables are somewhat lower (both the y intercepts and the exponents for the best-fitting power functions are 25%–33% smaller with the monochromatic than with the white stimuli), the functions for colored and white stimuli are highly correlated. The differences in the upper limit for normal sensitivities account for most of the differences in the neural versus sensitivity relationships. It seems that some mechanisms of ganglion cell loss affect visual thresholds with either colored or white stimuli, but the smaller dynamic range of thresholds with colored lights and the smaller population of detection mechanisms causes a larger relative loss in sensitivity for these stimuli.

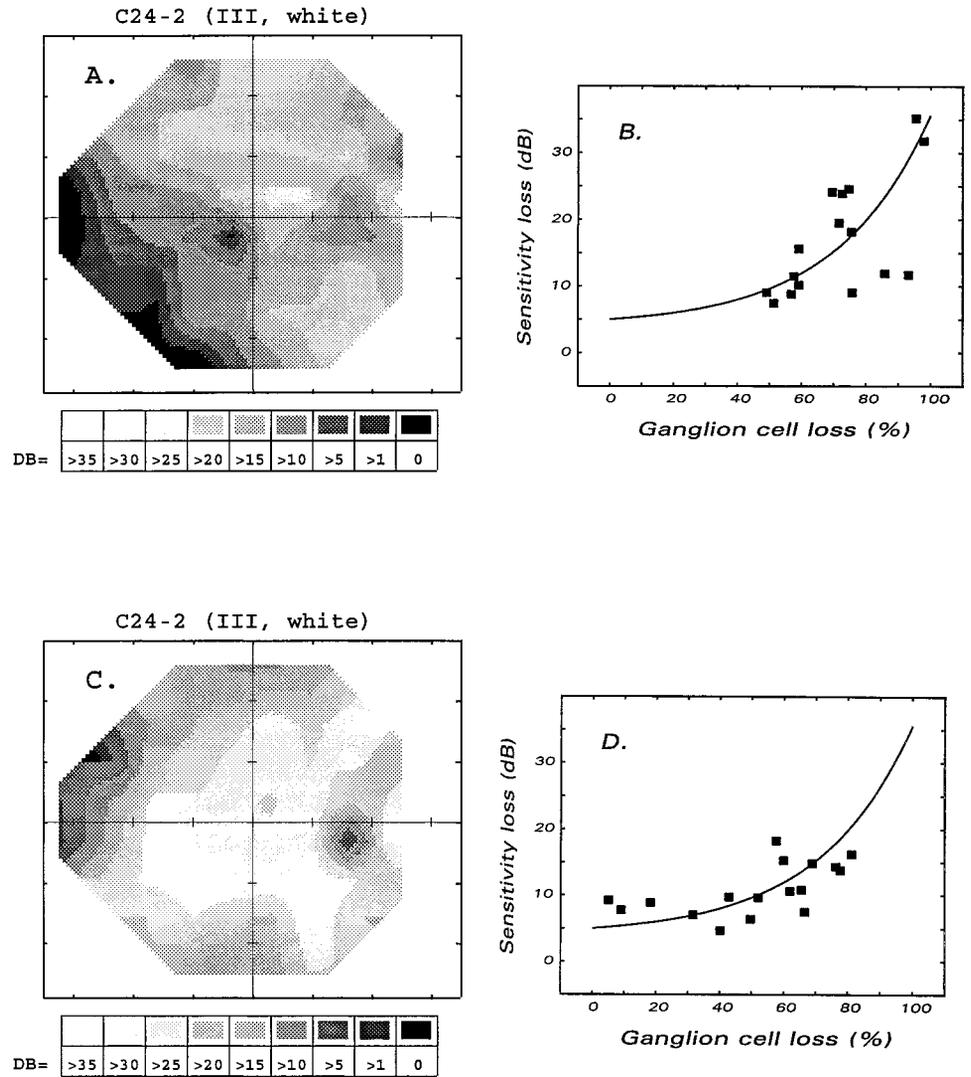


FIGURE 4. Examples of two individual subjects' losses of visual sensitivity as a function of their ganglion cell losses (*upper panels*: subject OHT-9; *lower panels*: subject OHT-8). The grayscale plots of visual fields of their treated eyes at the time of the histologic analysis are shown in the *left panels*, and the empiric sensitivity-neural relationships are presented in the *right panels*. The curve superimposed on the data for each subject is the best-fit power function from the group data (Fig. 3A). III, Goldmann III.

DISCUSSION

The principal finding of these experiments is that there is not a proportional relationship between visual sensitivity and ganglion cell losses caused by glaucoma.¹⁷ Only small changes in decibels of visual loss are associated with ganglion cell losses of less than approximately 50%, whereas for more advanced glaucoma the visual defects increase more systematically with ganglion cell loss. In addition, the general form and characteristic of the neural-sensitivity relationship appear to be essentially identical for white and colored stimuli when sensitivity values are normalized with respect to the expected levels for normal subjects.

In general, the results of the present investigations are a faithful replication of earlier studies of neural-sensitivity relationships using donated eyes of glaucoma patients.¹⁸ One of the most obvious similarities in the two investigations is in the substantial variability of the data (Fig. 3A). Even though many of the technical sources of variability may have been reduced by the use of a monkey model, either there are other unknown sources of experimental variability or there is an inherent variability in the sensitivity losses versus ganglion cell losses caused by glaucoma. Based on the concurrence of findings

from two studies, it is not certain whether improvements in the methodology for clinical perimetry might be able to produce more accurate estimates of the neural damage caused by glaucoma.

A second important finding, which is common to the investigations with both human and monkey subjects, is that the standard clinical protocol, using Goldmann III, white stimuli, provides a more accurate assessment of advanced than of mild losses of ganglion cells. The majority of the data from human eyes¹⁸ are for visual field defects associated with ganglion cell losses that were greater than 50%. The slope of the linear regression analysis of those data was 0.4 dB of sensitivity loss per percent of ganglion cell loss. This value is virtually identical with that found for linear regression of the data over the same range of ganglion cell losses from the present experiments. Thus, the results verify prior suggestions²⁹ that visual field defects measured with white light stimuli are a more accurate reflection of deep than mild neural damage. Specifically, these data indicate that visual threshold losses of greater than 15 dB with conventional Goldmann III, white stimuli, provide a reasonable estimate of the average amount of ganglion cell loss in excess of 50%.

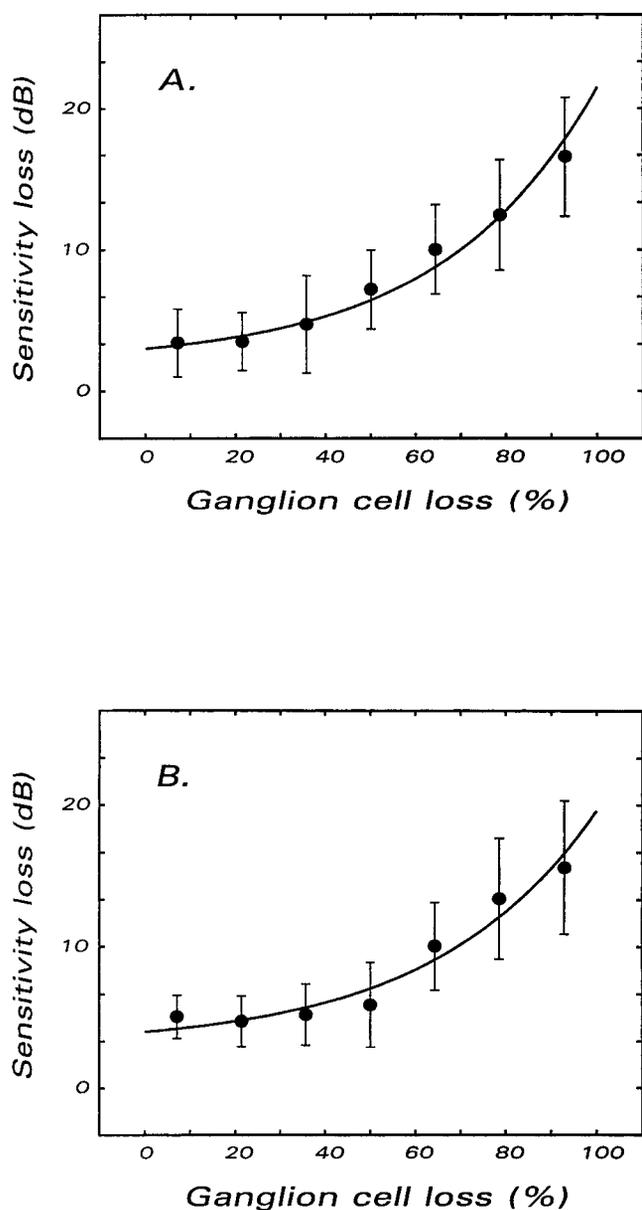


FIGURE 5. The loss of chromatic visual sensitivity as a function of the loss of ganglion cells caused by experimental glaucoma in macaque monkeys. (A) Sensitivity-neural relationship for a Goldmann V 460-nm stimulus to obtain perimetry thresholds mediated by a subpopulation of ganglion cells (i.e., the short-wavelength-sensitive mechanism). (B) Sensitivity-neural relationship for a Goldmann V 620 nm stimulus to obtain perimetry thresholds mediated by the color-opponent mechanism.

The use of a monkey model in these investigations allowed control of the stages of glaucoma at the times of histologic analyses, so that ganglion cell losses underlying mild, as well as deep, visual field defects could be assessed. The analysis of data over the relatively complete range of visual field defects showed that the neural-sensitivity relationship is best described by a curvilinear (power) function. However, in addition to an exponential factor, the best fitting function required a vertical shift of the function (see Fig. 3B). As a consequence, the value of the y-intercept represents a loss of sensitivity, approximately 6 dB, that usually precedes measurable ganglion cell loss.

Subsequent experiments have demonstrated that losses of sensitivity are not caused by interactive effects from elevated thresholds in adjacent, more highly affected, areas of the visual field.⁴⁴ It is not clear, however, to what extent this finding is a specific result of the experimental model. The monkey model is a high-pressure model,²¹ and the subjects were at various stages of actively progressing field defects.²² Thus, the most obvious explanation for the decreased sensitivity without neural loss is that the tissue was obtained at a time when neural function was compromised but before destruction of the ganglion cell body. This explanation is supported by anatomic studies of ganglion cells after optic nerve transection⁴⁵ or experimental glaucoma^{46,47} and would account for the unanticipated dissociation of the structure-function relationship. On the other hand, the same effect may be less likely for the ordinary human glaucoma patient because the time course is much slower,^{10,48} and because there is usually ongoing medical treatment to control intraocular pressure.^{3,5} Furthermore, in some cases patients may retain a normal number of optic nerve axons in the presence of a chronic intraocular pressure elevation.⁴⁹

It is also important that as it pertains to glaucoma diagnosis a 6-dB loss of sensitivity would not occur in the absence of neural damage, and the survival time of the cell bodies cannot explain an absence of correlation between ganglion cell losses and mild visual field losses. In some instances, nearly 70% of the ganglion cells at a test location were gone before the visual threshold was elevated by more than 15 dB (Fig. 4D). The absence of a significant correlation between sensitivity deficits and relatively small losses of ganglion cells has important implications for the early detection and diagnosis of glaucoma, which has been also recognized by clinical research on perimetry procedures.⁵⁰⁻⁵⁶ The clinical evidence for the poor sensitivity of threshold perimetry with standard white Goldmann III stimuli has been explained by either its lack of stimulus specificity for the mechanisms affected early in the course of the disease³⁴ or the fact that white light is a redundant stimulus for the various subpopulations of retinal ganglion cells.^{50,51}

The present experimental methods did not classify ganglion cells by their size or function, but two alternative perimetry stimuli, considered to isolate specific subpopulations of ganglion cells, were used for visual field measurements.²⁸ The empiric neural-sensitivity relationships for short-wavelength-sensitive mechanisms and color-opponent mechanisms (Fig. 5) are very similar to each other and, with appropriate scaling for differences in dynamic ranges, both are similar to the neural-sensitivity relationship found for white stimuli. Therefore, although perimetry with colored stimuli has a higher sensitivity for the early detection of glaucoma,³⁰⁻³³ perimetry threshold data with short- or long-wavelength stimuli do not provide a more accurate assessment of ganglion cell loss.

The results with these alternative perimetry stimuli were anticipated because perimetry thresholds and perimetry global indices with white and monochromatic stimuli are highly correlated.²⁸ On the other hand, because the neural-sensitivity relationships are essentially identical for both the short-wavelength and opponent-mechanism perimetry, it seems unlikely that the higher sensitivity for detecting glaucoma with monochromatic stimuli is based on the size-dependent susceptibility of ganglion cells to injury from glaucoma.^{18,46,47,57}

In conclusion, current perimetry regimens with either white or monochromatic stimuli do not provide a useful esti-

mate of ganglion cell loss until a substantial proportion has died. The findings of the present study are in concurrence with the original studies of Quigley et al.¹⁸ and emphasize the importance of continued investigations into psychophysical methods for more accurate and reliable determinations on the direct ocular effects of glaucoma. Such methods would be especially important in providing a realistic appraisal of the effectiveness of treatments in preventing the progression of neural damage and blindness from glaucoma.

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