Neural Losses Correlated with Visual Losses in Clinical Perimetry

Ronald S. Harwerth,[1] *Louvenia Carter-Dawson,*[2] *Earl L. Smith III,*[1] *George Barnes,*[3] *William F. Holt,*[3] *and Morris L. J. Crawford*[2]

Purpose. The validity of clinical perimetry for evaluation of the pathology of glaucoma is based on correlated losses in retinal ganglion cells and visual sensitivity, but procedures to quantify neural losses from visual field defects have not been developed. The purpose of the present study was to investigate the neural and sensitivity losses from experimental glaucoma to establish the framework for a quantitative model for the structure–function relationships of standard clinical perimetry.

Methods. Perimetry, by behavioral testing, and retinal histology data were obtained from rhesus monkeys with significant visual field defects caused by experimental glaucoma. Ganglion cell densities were obtained from sections of retina that corresponded to 16 perimetry test locations. Perimetry sensitivity as a function of ganglion cell density at corresponding retina/visual field locations was analyzed.

Results. The structure–function relationships were linear on log–log coordinates, with parameters that varied systematically with eccentricity. The slope value varied from 1.25 dB/dB at 4.2° from fixation to a value of 2.32 dB/dB at 24° from fixation, whereas the intercept value varied from −25.2 dB to −55.7 dB over the same range of eccentricities. The structure–function relationships produced a model to predict the ganglion cell density underlying a given level of visual sensitivity and location in the visual field. The model, with no free parameters, produced an accurate and relatively precise quantification of retinal ganglion cell losses caused by experimental glaucoma in monkeys. However, because the early detection of glaucoma is limited by intersubject variability, ganglion cell losses of 40% to 50% were necessary before visual sensitivity losses exceeded the normal 95% confidence limits.

Conclusions. With retinal eccentricity as a factor, the neural losses from glaucoma are predictable from visual sensitivity measurements by clinical perimetry. The relationships derived from experimental glaucoma in monkeys also accurately predict the rate of age-related losses of retinal ganglion cells in humans, based on the normative perimetry data for age-related reductions in visual sensitivity. The success of the model in this study suggested that it is potentially applicable to the clinical interpretation of the state of glaucomatous optic neuropathy. (*Invest Ophthalmol Vis Sci.* 2004;45:3152–3160) DOI:10.1167/iovs.04-0227

Computer-automated perimetry, using white-light test targets that are superimposed on a white background, has become the standard test for the assessment of vision loss and the evaluation of clinical stages of glaucoma.[1–6] Although the use of perimetry for the initial diagnosis and follow-up of glaucoma is generally accepted,[7–9] documentation of the stage of the disease by visual field defects is not straightforward because visual losses are secondary to the primary pathologic effect of ganglion cell dysfunction and/or death. Thus, the clinical interpretation of perimetry data requires an accurate translation between the level of visual sensitivity and the population of retinal ganglion cells at each of the sites tested. However, the quantitative structure–function relationships required for translating visual losses to neural losses have not been determined and, therefore, the purpose of the present investigation was to develop a model to predict point-wise neural losses from clinical perimetry measurements of visual sensitivity across the visual field.

The primary reason that a quantitative structure–function relationship for clinical perimetry has not been developed is because of the considerable imprecision and inaccuracy in the relationship between losses of visual sensitivity and losses of retinal ganglion cells that have been reported previously.[10–13] For example, the initial studies by Quigley et al.[9] indicated that, on average, statistically significant visual field defects required neural losses of 20% to 50%, depending on the retinal eccentricity, but for any given level of neural loss there was a very large range of visual field defects. Subsequent studies, using a model of experimental glaucoma in macaque monkeys,[10] confirmed those results and also demonstrated that the structure–function relationship was more systematic, although still quite variable, when the neural losses exceed 50%. More recently, Kerrigan-Baumrind et al.[12] reported on a study of a relatively large number of glaucoma patients that showed a very low point-wise correlation between visual sensitivity and ganglion cell losses, but the relationship was improved if global measures of sensitivity, such as average sensitivity loss or mean deviation (MD), were used to assess vision loss.

There are many factors that may have limited the accuracy and precision of translating clinical visual fields to ganglion cell losses, but two seem especially important: 1) the appropriate measurement scales for sensitivity and neural losses; and 2) the inclusion of retinal eccentricity as an independent parameter. First, the prior studies have presented the data on scales in different units, for example, visual loss on a decibel (dB) scale and the neural loss as a percentage. The use of unequal scales for sensory and neural losses affects the accuracy of the relationship, but the correct scale is not certain and both linear and logarithmic transformations have been advocated.[13–16] The use of a log–log coordinate system has strong theoretical support

From the [1]College of Optometry, University of Houston, Houston, Texas; [2]Department of Ophthalmology and Visual Science, The University of Texas–Houston, Houston, Texas; and [3]Department of Pharmacology, Alcon Research, Ltd., Fort Worth, Texas.

Supported by Alcon Research, Ltd., Fort Worth, Texas; Research to Prevent Blindness, New York, New York; the Vale-Asche Foundation to the Herman Hospital Eye Fund, Houston, Texas; National Eye Institute Grants EY01139, EY03611, and EY10608 and Core Grants EY07551 and EY11545; and a John and Rebecca Moores Professorship from the University of Houston, Houston, Texas.

Submitted for publication March 1, 2004; revised May 5, 2004; accepted May 10, 2004.

Disclosure: **R.S. Harwerth**, None; **L. Carter-Dawson**, None; **E.L. Smith III**, None; **G. Barnes**, None; **W.F. Holt**, None; **M.L.J. Crawford**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Ronald S. Harwerth, 505 J. Davis Armistead Building, University of Houston, 4901 Calhoun Boulevard, Houston, TX 77204-2020; rharwerth@uh.edu.

Investigative Ophthalmology & Visual Science, September 2004, Vol. 45, No. 9

from the statistical description of probability summation for the detection of a stimulus that is imaged on a retinal area with multiple detectors.[17–21] The fundamental principle of probability summation is that an observer will detect a stimulus whenever at least one of the potential detectors in the population actually detects the stimulus. By this principle, visual thresholds are not determined by linearly summed responses of all the detectors in the total population, but rather by nonlinear pooling among neural detectors.

The general relationship for sensory versus neural substrates derived from probability summation is an exponential function of the number of detectors and the probability of detection for each of the available mechanisms. It is more desirable to have linear relationships for the quantitative model of the structure–function relationship for clinical perimetry, and the exponential function for visual sensitivity versus ganglion cell density becomes linear via logarithmic transforms on both variables. The utility of logarithmic scaling has been confirmed for experimental glaucoma in monkeys,[13] which demonstrated that the empiric relationship between visual sensitivity, in dB (the threshold value from a given test location for the 24–2 program of the Humphrey Visual Field Analyzer; Carl Zeiss-Meditec, Dublin, CA), as a function of ganglion cell density, in dB (10-times the logarithm of the histologic count of ganglion cells at the corresponding retinal location), was well-described by linear regression. The parameters for the linear regression (i.e., a *y*-intercept value of near zero and a correlation coefficient of 0.97) indicated that the general relationship was accurate; although the accuracy was accomplished by compression of the neural losses associated with small losses of visual sensitivity.

Although the data for experimental glaucoma demonstrate that the utilization of measurement scales based on specific psychophysiological links can improve the accuracy of the structure–function relationship, the data transform does not improve the precision of the empiric relationship. The primary source of significant imprecision is likely to be related to the second factor in the translation of clinical visual fields to ganglion cell losses, that is, variations in the structure–function relationship with retinal eccentricity. Eccentricity factors have not been considered in previous reports of neural-sensitivity losses from glaucoma, although in retrospect, retinal eccentricity as an independent parameter should have been intuitive. The inclusion of an eccentricity parameter for glaucomatous vision loss could have been anticipated because both of the variables of the structure–function relationship are eccentricity dependent. For example, studies of retinal ganglion cell density with retinal eccentricity in both humans[22–24] and monkeys[25–28] have documented nonuniform distributions with the highest concentrations of cells near the fovea and a ten-fold reduction in cell density in the midperiphery. Similarly, perimetric sensitivity is highest near the fovea and falls in the midperiphery by a factor of approximately 5,[29–32] and in this case, intersubject variability of sensitivity also varies, being lowest in the central field with systematic increases across the peripheral visual field.[33,34]

Based on these variations in normal neural and sensitivity functions, it seems reasonable that point-wise predictions of neural losses from clinical perimetry measurements of visual sensitivity should include a parameter for retinal eccentricity, and for each eccentricity the neural and visual measurements should be specified in logarithmic units. Thus, the present studies were undertaken to develop a quantitative model for the structure–function relationship for clinical perimetry by the incorporation of these factors. The data to construct the model were an extension and re-analysis of data from earlier investigations of the ganglion cell losses underlying visual field defects from experimental glaucoma in macaque monkeys.[11,13] An empiric model was developed by linear regression analysis, which provided accurate and precise estimates of ganglion cell densities from perimetry sensitivity measures. In addition, in an application to clinical data, the model also accurately predicted the normal age-related loss of retinal ganglion cells from the normal age-related reduction in perimetric visual sensitivity and, therefore, even though developed from data of experimental glaucoma, the results also should be generally applicable to clinical patients. Some of the results of these studies have been presented briefly elsewhere (Harwerth RS, et al. *IOVS* 2003;44:ARVO Abstract 1040) and portions of the raw data have been published in other forms.[13]

Materials and Methods

Subjects

The subjects for the investigations were 16 adult rhesus monkeys (*Macaca mulatta*) that had been behaviorally trained for standard clinical perimetry measurements. The experimental and animal care procedures were reviewed and approved by the Institutional Animal Care and Use Committees of the University of Houston and the University of Texas–Houston. The use of animals for these experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Experimental glaucoma was induced unilaterally in 14 of the monkeys and bilaterally in the other two. Their intraocular pressures were elevated by argon laser treatment of the trabecular meshwork, using energy levels that destroy the trabecular meshwork and obliterate Schlemm's canal in the vicinity of the laser burn. Multiple sessions, separated by several weeks, were used to treat the full 360° of the trabecular meshwork to create sustained elevated pressures that were generally greater than 40 mm Hg. Other details of the protocol for laser treatments and intraocular pressure measurements have been published previously.[35] The protocol for bilaterally treated animals required close monitoring of visual fields to terminate the experiment before bilateral blindness occurred. In both animals the visual fields defects were asymmetric with only mild visual field losses in one eye at the time the retinal tissues were collected.

Behavioral Perimetry

The visual field defects caused by experimental glaucoma were assessed by static threshold perimetry using standard clinical instrumentation. A Humphrey Field Analyzer (HFA) was attached to a primate-testing cubicle and the monkeys were trained to fixate and perform a psychophysical detection task that was essentially the same as used for standard clinical perimetry. The testing procedures, which have been described in detail,[32] provided highly reliable data throughout the course of experimental glaucomatous neuropathy. For the present experiments, each monkey's visual fields were followed until the development of reliable visual defects, and then retinal tissues were collected for histologic analysis.

Histological Analysis

Within 2 days after the final visual fields test, the monkey was deeply anesthetized, the eyes were enucleated, and the posterior segments were fixed for histology. Retinal tissue samples were collected from retinal locations that correspond to 16 of the test locations for the C24–2 program of the Humphrey Field Analyzer. The retina samples were sectioned and stained for quantification of ganglion cell densities using methods for tissue preparation and cell measurements that have been published.[13] Data from all 16 test locations were collected for 11 of the subjects, but only partial data (two–six samples) were available for five of the unilaterally treated subjects.

Results

The general approach to the development of a model to predict ganglion cell densities from visual sensitivity measure-

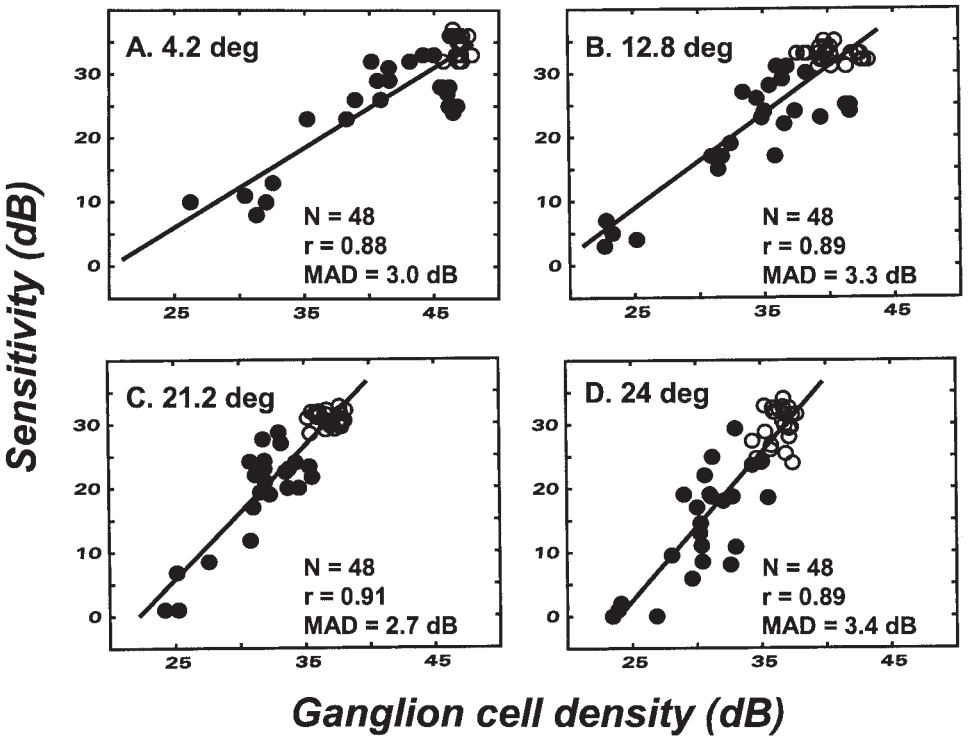

FIGURE 1. Structure–function relationships for clinical perimetry. (**A–D**) The relationships between visual sensitivity and ganglion cell density are presented for four visual field eccentricities (indicated on the graphs). The data are from six monkeys with unilateral experimental glaucoma for the control and treated eyes (*open* and *closed symbols,* respectively). The best-fit functions by linear regression are shown for each set of data, with the correlation coefficients (*r*) and mean absolute deviations (MAD) of the linear regression indicated on each graph. The parameters for the linear functions and other details of the structure–function relationships are presented in Table 1.

ments was to analyze the data from a selected group of monkeys to develop the parameters for the model and then test the model against data from other animals that were not in the initial set. Thus, the parameters for the model were derived from the data for six monkeys with stable, unilateral visual field defects that covered a range of mild to severe sensitivity losses. The correlated visual and neural data from the 16 sample locations for the control and treated eyes were analyzed for each eccentricity (designated by the radius from fixation, in arcdeg). The data for these six monkeys, presented in Figures 1A–D, were scaled in equivalent decibel (dB) units, with the visual sensitivities from perimetry measurements in standard dB units and the ganglion cell densities converted to dB units by multiplying the logarithm of the histological cell count by 10. In each plot, the data for the control and treated eyes are represented by open and solid symbols, respectively. The parameters for the structure–function relationships were determined by the best-fit linear regression of the sensitivity to the ganglion cell data in these log–log coordinates. In each case, the linear function provided an excellent description of the data, as is shown by the two statistical indices (*r*, the correlation coefficient, and MAD, the mean absolute deviation) that are noted on each plot. The low MAD's (i.e., measures of the goodness-of-fit of the data that are derived from the average absolute difference between the empiric sensitivity and the sensitivity predicted by the linear function) and high correlation coefficients indicate a high precision and accuracy of the linear relationships. Consequently, these data produce the framework for a model to predict ganglion cell densities from the corresponding visual sensitivities by clinical perimetry.

The numerical values for the sensitivity–neural parameters are presented in Table 1. The first three rows of the table present the data from the control eyes that describe a normal decrease in ganglion cell density of approximately 10 dB over the sampled range of eccentricities and a normal decrease in visual sensitivity of approximately 5 dB. The next three rows present data on the effects of visual sensitivities from reduced ganglion cell densities for the treated eyes. The parameters for linearity between sensitivity and neural losses (both in dB units) show an increase in slope and a decrease in the intercept with eccentricity, while the coefficient of determination remains essentially constant, with approximately 80% of the variance explained by the linear functions. The final two rows of Table 1 present an estimate of the minimum density of ganglion cells required for a sensitivity measurement by the clinical procedure. The minimum cell density, below which

TABLE 1. Eccentricity-Dependent Parameters for Structure–Function Relationships for Standard Clinical Perimetry

	Eccentricity			
	4.2°	**12.8°**	**21.2°**	**24°**
Normal density (cells/mm^2)	48,982 ± 4,906	11,188 ± 1,290	4,986 ± 1,016	4,458 ± 898
Normal density log (cells/mm^2)	46.9 ± 0.4	40.2 ± 0.6	36.9 ± 0.9	36.4 ± 0.9
Normal sensitivity (dB)	34.58 ± 1.36	32.79 ± 2.10	29.87 ± 2.96	29.42 ± 3.20
Slope (dB/dB)	1.25	1.47	2.08	2.32
Y-intercept (dB)	−25.2	−32.3	−46.2	−55.7
Coefficient of determination	0.76	0.77	0.83	0.79
Cell density @ 0-dB sensitivity (cells/mm^2)	100	160	170	250
Percent normal cell density @ 0-dB sensitivity	0.2%	1.4%	3.4%	5.6%

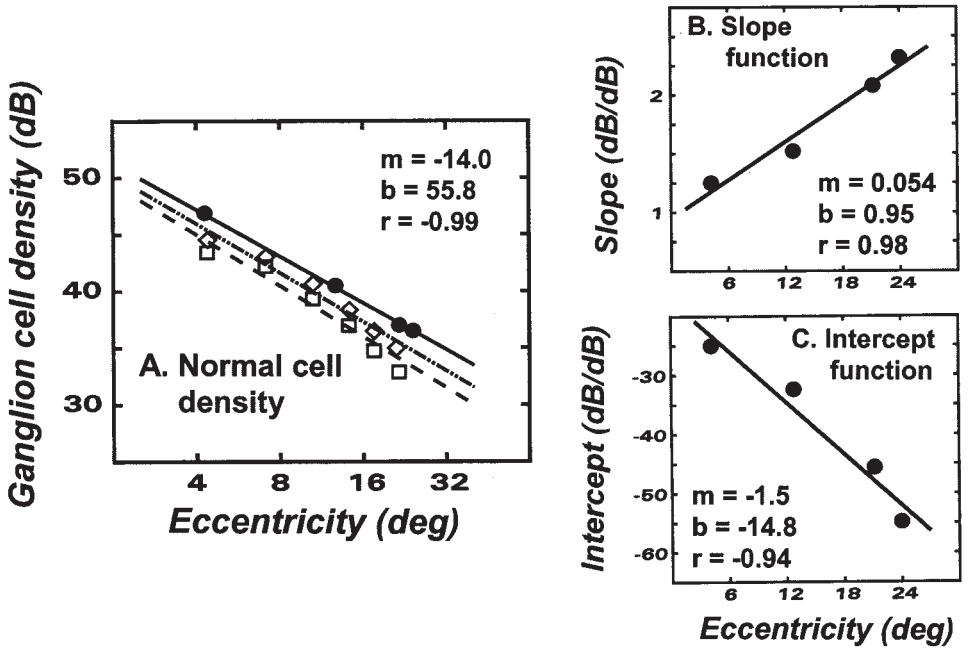

FIGURE 2. Eccentricity functions for the parameters of the structure–function relationships. (**A**) The normal retinal ganglion cell density as a function of retinal eccentricity (note that eccentricity is on a logarithmic scale). Data from the control eyes of monkeys are represented by the *filled symbols* and *solid line*. The parameters of the fitted linear function (*m*: slope, *b*: intercept, and *r*: correlation coefficient) are shown as an inset. For comparison to the monkey's data, Curcio and Drucker's[23] data from young (*diamonds* and *dot-dash line*) and aged (*squares* and *dashed line*) human subjects are presented. (**B**, **C**) The slope and intercept parameters for the linear structure–function relationships (see Fig. 1) as a function of retinal eccentricity. The parameters of the fitted linear functions (*m*: slope, *b*: intercept, and *r*: correlation coefficient) are shown as an *inset* on each graph. The three functions (**A**–**C**) constitute the model for predicting ganglion cell loss from visual field data.

the perimetric sensitivity is zero, varies with eccentricity from 0.2% near fixation to 5.6% in the peripheral nasal field. Thus, the point of failure for clinical perimetry is eccentricity-dependent, with an inverse relationship to the normal ganglion cell density.

The data presented in Figure 1 and Table 1 demonstrate that the relationships between sensitivity and ganglion cell losses are systematic at any given eccentricity and that the variables of the neural–sensitivity functions vary across eccentricities. The eccentricity effects for the normal retinal ganglion cell density and the slope and intercept parameters of the structure–function model are illustrated in Figure 2. In each case, the appropriate choice of the scale for the ordinate creates a linear relationship, with the normal ganglion cell density varying linearly with eccentricity (Fig. 2A) when both variables are in logarithmic units, whereas the slope (Fig. 2B) and intercept (Fig. 2C) parameters vary with eccentricity in linear units. The correlation coefficients were 0.94, or higher, indicating an accurate relationship for each of the model's parameters for prediction of the amount of neural loss from a visual sensitivity measurement. Accordingly, the parameters for the linear functions, noted on each graph of Figure 2, provide the quantitative linear model for the structure–function relationship for clinical perimetry.

A test of the model is shown by the data in Figure 3A, which presents the point-by-point relationship between the measured, histological ganglion cell density and the ganglion cell density predicted from visual sensitivity by the functions shown in Figures 2B and 2C. Data are presented from eight monkeys that were not used to develop the model parameters; two of the monkeys were bilaterally treated, and only partial data were available for five of the unilaterally treated subjects. The data for each retinal eccentricity are represented by different symbols, with data from control eyes shown by open symbols and data from treated eyes by filled symbols. A solid line has been superimposed on the data to delineate the model's optimal performance of a unity correlation between predicted and measured cell densities. It is apparent that the deviations of empiric data from the one-to-one line are nonsystematic, with a MAD of 1.84 dB and a coefficient of determination of 0.77. Hence, the statistical indices demonstrate that the model provides relatively precise and accurate predictions of ganglion cell density from visual sensitivity measurements for both the control and treated eyes of monkeys with experimental glaucoma. The densities for the control and treated eyes follow a single function and overlap considerably, even though the data for different retinal eccentricities fall into separate ranges that are set, primarily, by the normal cell densities at each eccentricity. For example, the test locations near fixation (4.2 arcdeg from fixation; circles in Fig. 3A) have the highest normal cell densities (open circles) and the cell densities associated with loss of visual sensitivity (filled circles) are at the higher end of the scale. Consequently, although the data confirm that the calculation of ganglion cell density from visual sensitivity requires an eccentricity factor, the different ranges of effects complicate the analysis of normal versus abnormal ganglion cell densities. For this reason, the clinical interpretation of the data may be facilitated by following the precedent of total deviation plots for visual sensitivity used in clinical perimetry, that is, the relative loss of ganglion cell density with respect to the expected normal density at each retinal location.

The quantitative performance of the structure–function model to predict relative losses of retinal ganglion cell density during glaucomatous neuropathy is illustrated in Figure 3B. For this test of the model, the predicted ganglion cell losses were derived from the difference between the normal cell density at a given eccentricity (Fig. 2A) and the cell density based on the visual sensitivity data and the test eccentricity (Figs. 2B and 2C). Data for the empiric (measured) values of cell losses for the treated eyes were determined by the differences in histologic cell counts for retinal samples at corresponding locations in the treated and control eyes. For the control eyes, the measured losses were the difference between the histologic cell count for a given sample and the mean normal cell density at the same eccentricity (Table 1). Data are presented from all the unilaterally treated monkeys in a general format of data presentation that is the same as Figure 3A, with the data for each retinal eccentricity represented by different symbols (control eyes, open symbols; treated eyes, filled symbols), and a solid line to delineate the model's optimal performance of a unity correlation between predicted and measured cell losses.

The re-analysis of the data as cell-loss functions accomplished normalization across visual field locations, with the

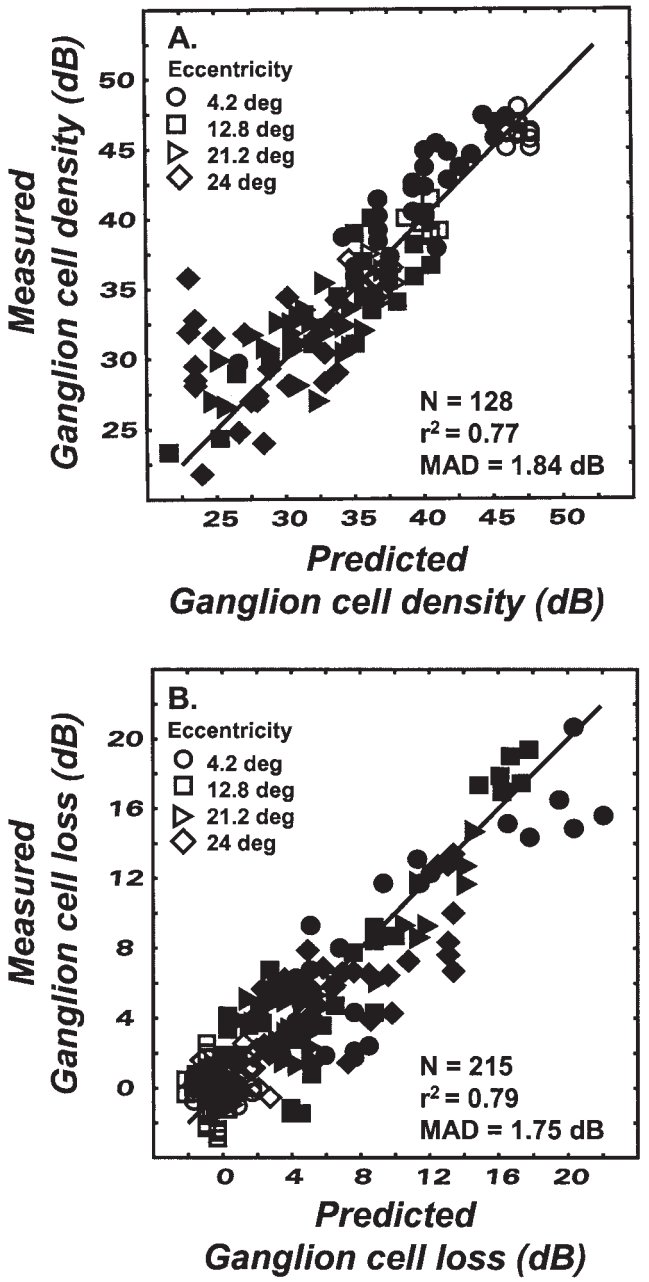

FIGURE 3. Point-wise predictions of retinal ganglion cell density from perimetric measurements of visual sensitivity. (**A**) The point-by-point comparison of ganglion cell density measured by histology versus the ganglion cell density predicted from the corresponding visual field sensitivity. Data for each eccentricity are represented by the symbols indicated in the *inset*, with *open* and *filled symbols* representing the control and treated eyes, respectively. The *line* superimposed on the data represents the one-to-one relationship between measured and predicted ganglion cell densities, and the r^2 and MAD values, shown on the graph, were calculated from the variance of the data with respect to the one-to-one correlation. (**B**) The relative measured loss of retinal ganglion cells (difference in histological cell counts for the control and treated eyes) versus the predicted loss of ganglion cells (difference in the predicted normal cell density and the cell density predicted from the visual sensitivity). The conventions for data symbols and statistical analysis data are the same as for (**A**).

data for the control eyes clustered near the origin of the function and the data for the treated eyes distributed across similar ranges of cell loss at all eccentricities. It is also important that the extra step of normalization did not affect the accuracy or precision of the model, as indicated by the clustering of the data around the solid line (Fig. 3B) representing a one-to-one relation between measured and predicted ganglion cell densities. The degrees of accuracy and precision are also indicated by the goodness-of-fit statistics for a unity correlation (i.e., the MAD of 1.75 dB and a coefficient of determination of 0.79) that were similar to the non-normalized data (Fig. 3A). It is important to note, however, that although the statistics indicate that the one-to-one line accounts for a high proportion of the variance, a larger proportion of the data fall below the line than above. The greater predicted than measured cell loss is consistent with sensitivity losses caused by cell dysfunction, in addition to cell loss, which has been suggested as a component of experimental glaucoma.[11,16]

The final results of the model for structure–function relationships in clinical perimetry are illustrated in Figure 4. The diagram is based on model calculations for the relative losses of ganglion cells underlying the losses of visual sensitivity for test locations either near fixation (4.2° eccentricity) or more peripheral (24° eccentricity). Each function represents the full extent of visual field defects from the earliest defect to the limit of visual sensitivity measurement with deeper losses. To illustrate the structure–function relationships for early glaucoma, the origins of the functions have been enclosed in boxes that represent the 95% confidence limits for cell density or sensitivity losses at each eccentricity. In both cases, the confidence limits derived from intersubject variability of ganglion cell densities are much smaller than the confidence limits for sensitivity losses, and the confidence limits for both variables are considerably smaller for the central test sites (cross-hatched area) compared to the peripheral test sites (oblique hatching). However, because of the difference in the slopes of the functions, the initial appearance of clinically significant visual field defects occurs with similar amounts of ganglion cell loss at either eccentricity. For example at an eccentricity of 24°, the 95% confidence limit for visual sensitivity is 6.4 dB, which

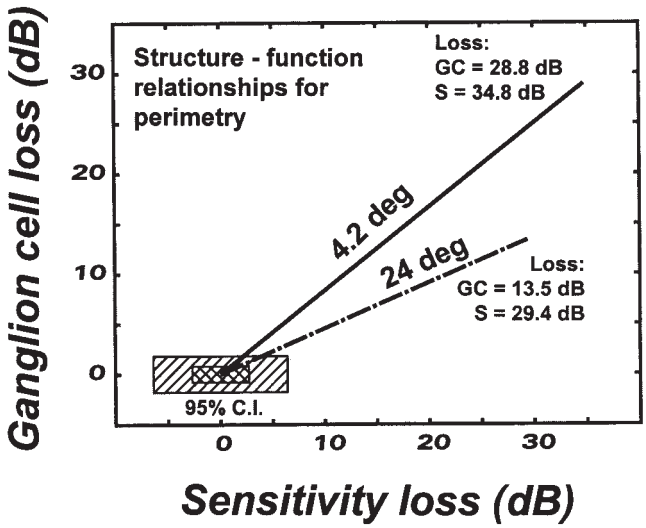

FIGURE 4. The quantitative model for structure–function relationships for clinical perimetry. The *lines* represent the calculations for the relative losses of ganglion cells underlying the losses of visual sensitivity for test locations near fixation (4.2° eccentricity) or more peripheral (24° eccentricity). The *boxes* at the origin of the functions represent the 95% confidence limits of neural and visual losses for the central (*cross hatching*) or peripheral (*oblique hatching*) functions. The *numbers* placed near the upper limits of the functions represent the relative loss in ganglion cells (GC) that results in the maximum detectable loss of sensitivity (S) with the standard perimetry stimulus.

corresponds to a ganglion cell loss of approximately 3 dB, or 50% loss of ganglion cells before the visual field defect becomes statistically significant. In comparison, at the central test location, to exceed the 95% confidence limit of 2.7 dB requires a ganglion cell loss of 2.2 dB, or a 40% loss of ganglion cells.

The differences in the slopes of the function across retinal eccentricities not only explains the similarities in cell losses underlying early visual field defects, but also accounts for the substantial differences in ganglion cell densities at the upper limit of measurable visual sensitivity by standard clinical perimetry. The slopes of the functions are attributable to the variation in normal ganglion cell density from central to peripheral retina and, therefore, the limit of measurable visual sensitivity loss represents considerably greater losses of retinal ganglion cells in the central retina than in the periphery. These differences are detailed in Figure 4 by the relative losses in ganglion cell density and visual sensitivity, noted beside each function, that are associated with immeasurable visual sensitivity using the standard, Size III, perimetry test target. It is especially interesting that the clinical procedure requires only one-fifth of a percent of normal ganglion cells for central field measurements, but >5% normal ganglion cells for peripheral visual field measurements (Table 1). Thus, for the full range of visual field defects caused by glaucoma, the functions illustrated in Figure 4 demonstrate the general importance of including retinal eccentricity as an independent parameter in quantifying the structure–function relationship for clinical perimetry.

Discussion

The intent of the present study was to develop a model for the accurate and precise prediction of the structural (neural) defects caused by glaucoma from measurements of visual function by clinical perimetry. Although the purpose of the investigation was to derive a general clinical application for structure–function relationships, it was a necessary first-step to conduct controlled investigations on animal subjects to determine the feasibility and framework. The research method involved the induction of experimental glaucoma in macaque monkeys to produce a progressive optic neuropathy that was assessed by behavioral perimetry. Our previous investigations,[11,13,35–40] using these methods, have demonstrated that experimental glaucoma is an excellent preparation for evaluating the psychophysical and histopathological effects associated with the death of retinal ganglion cells. For the present investigation, the research method has important benefits in reducing experimental variability for measurements of both visual and neural effects which, if not controlled, would have obscured the critical trends that define the model. Examples of the principal controls of variability are: 1) experimental glaucoma generally is unilateral, which allows sensitivity versus neural losses to be assessed by differences between the treated and control eyes of a single subject; 2) the condition may be allowed to progress to analyze neural–visual relationships from the full range of glaucomatous neuropathy; and 3) at any stage, monkeys produce highly reliable perimetry data because of daily practice with rigorous behavioral control and the final visual fields data and collection of histologic tissue are essentially simultaneous, with the retinal tissue fixed and processed immediately. Therefore, in contrast to the experimental difficulties related to obtaining accurate perimetry data and postmortem retinal tissue from aged patients, the methods and control afforded by experimental glaucoma provided the quality of data that was required to develop a quantitative structure–function model for standard clinical perimetry.

The structure–function model is a linear regression for a point-wise analysis of the degree of retinal ganglion cell loss from single measurements of visual sensitivity by standard clinical perimetry. The model, with no free parameters, produces relatively precise and accurate quantification of retinal ganglion cell losses caused by experimental glaucoma in macaque monkeys. In principle, the model could be applied directly to clinical patients on the basis of the close similarities in the anatomic and functional properties of the monkey and human visual systems,[41] including clinical perimetry.[32] However, none of the prior studies have directly compared the structure–function relationships for visual field defects caused by the loss of retinal ganglion cells and, therefore, an empiric test of the present model against data from humans is important. Although the most direct test would be with glaucoma patients, those data were not available to us at the time and, instead, the model was tested against published data from humans on the normal variation of ganglion cell density with eccentricity and the normal age-related losses in retinal ganglion cell density.

One of the most fundamental variables of the model is the expected normal ganglion cell density at any location in the retina. For the control eyes of monkeys, the ganglion cell density as a function of eccentricity is linear in log–log coordinates (see Fig. 2A, filled circles) with a coefficient of determination of 0.98. The function for ganglion cell density versus eccentricity for humans is similar, as demonstrated by the data from Curcio and Drucker[24] for subjects in two age groups. These data are presented in Fig. 2A, with the data for young subjects (age, 27–37 years) shown by the open diamonds and a dot-dash line and the data for aged subjects (age, 66–82 years) shown by the open squares and a dashed line. The parameters for the fitted functions for the monkey and human data are very similar, that is, the slopes for all three functions are within 1 dB/deg (monkeys: −14.0 dB/deg; young humans: −14.4 dB/deg; aged humans: −15.0 dB/deg) and the intercepts are within 2 dB (monkeys: 55.8 dB, young humans: 54.6 dB; aged humans: 54.0 dB). The small displacements of the data, indicated by the intercept values, are consistent with the normal age-related loss of retinal ganglion cells.[12,42,43] The human-equivalent ages of the monkeys were 24–28 years, slightly younger than the young humans for Curcio and Drucker's study, and they show the highest ganglion cell densities at each eccentricity. The data for the two groups of human subjects are then ordered by systematic reductions with increasing age. It is also important to note that the age-related changes are essentially uniform across eccentricities, that is, there are no regional differences, which is also consistent with other published data for humans.[42,43] Thus, the function for the variation in retinal ganglion cell density with eccentricity can be applied to clinical data, although to maintain the accuracy of the model for the expected normal ganglion cell densities there will need to be a factor for normal age-related cell losses.

The rate and magnitude of the age-related ganglion cell losses were investigated further by determining whether the model that was derived from monkeys could accurately predict the normal age-related losses of retinal ganglion cells in humans. This evaluation may be considered a stringent test of the model because the normal loss of ganglion cells over a lifetime is relatively small, <50% (Fig. 5). The elemental data for the test were the age-related losses of visual sensitivity that are incorporated into the HFA StatPac (Carl Zeiss-Meditec) for statistical analysis of clinical perimetry data.[30,31] The perimetry data for the expected normal visual sensitivity as a function of age at each of four eccentricities are presented in Figure 5A. The characteristics of these data are well known and, as expected, they show an overall reduction in sensitivity with increasing eccentricity and an age-related loss of sensitivity that is more rapid for more peripheral than central test locations (i.e., slopes = −0.06 dB/year at 4.2° eccentricity, −0.07 dB/

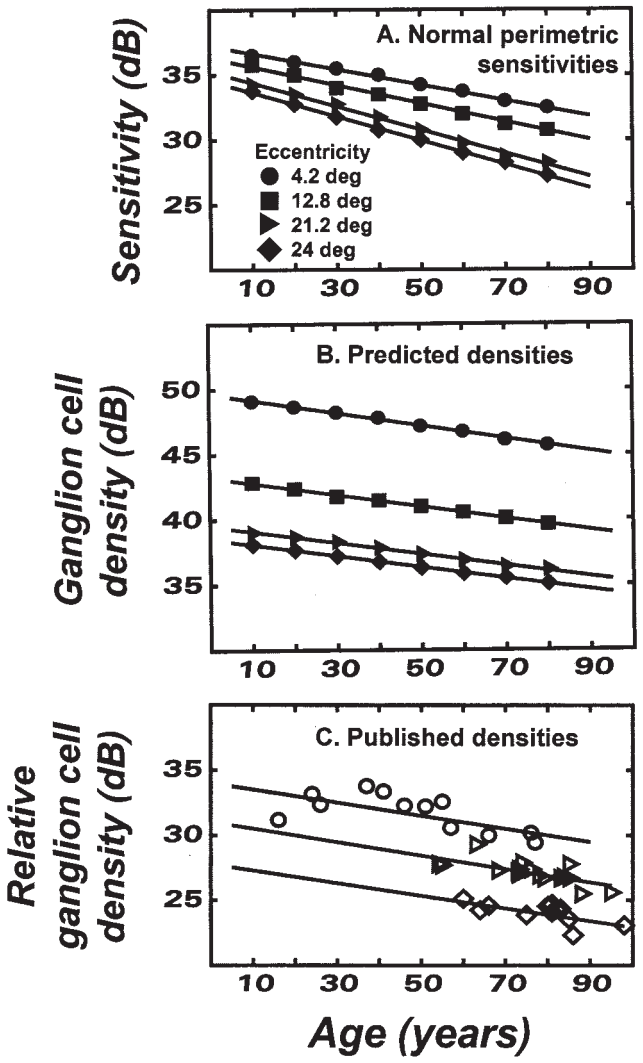

FIGURE 5. The normal age-related losses in retinal ganglion cells in humans predicted from age-related losses in visual sensitivity. (**A**) The data represent the normal age-related reduction in visual sensitivity for the four visual field eccentricities indicated on the graph. The data were obtained from the HFA StatPac database. (**B**) The predicted age-related losses of retinal ganglion cells that are predicted from the age-related losses in visual sensitivity (**A**) using the structure–function relationships illustrated in Figure 2. (**C**) The histological ganglion cell losses as a function of age from three studies, Harman et al.,[43] (*circles*), Kerrigan-Baumrind et al.,[12] (*triangles*), and Blanks et al.,[42] (*diamonds*) with rates of age-related losses that are similar to the predicted rates in (**B**).

year at 12.8° eccentricity, and −0.09 dB/year at 21.2° or 24° eccentricity). The results of the model, using the normative sensitivity values as input data to predict ganglion cell densities, are presented in Figure 5B. It is apparent that, although the age-related functions are separated by the normal variation in ganglion cell density with eccentricity, the slopes of the functions have become uniform across eccentricities. Thus, the model predictions are that a constant proportion of retinal ganglion cells are lost each year as a part of normal aging (about −0.046 dB/year) and that the rate of loss is similar at all retinal locations. Both predictions are consistent with published data. For example, the rate of age-related ganglion cell losses that were predicted from visual sensitivities (Fig. 5B) is remarkably similar to the rate found in histologic studies. This result is illustrated by data from three recent studies,[12,42,43] presented in Figure 5C, for the normal retinal ganglion cell density (in dB units) as a function of age. The slopes of the fitted functions, which vary between −0.047 and −0.052 dB/year, are compatible with the rate of cell loss predicted by the model. Thus, these data demonstrate the potential application of the structure–function model and also provide confirmation that the age-related losses in visual sensitivity are primarily caused by retinal neural losses rather than preretinal light losses from crystalline lens opacities or pupillary miosis.[44]

The combined evidence obtained from accurately predicting retinal ganglion cell losses from both experimental glaucoma in monkeys and normal ageing in humans provides strong proof of the principle for a quantitative model for structure–function relationships for standard clinical perimetry. Obviously, the final formulation of the model will require data from human glaucoma patients, but the present approach of behavioral control in animals and group data for humans was necessary to reduce experimental variability and define the basic parameters of the model. However, some forms of variability cannot be eliminated. For example, visual sensitivity losses must precede ganglion cell death and psychophysical measures include a component of cell dysfunction as well as cell death. In this respect, visual sensitivity may represent the truer evaluation of functional status because it includes both components.

Another source of inherent variability that cannot be reduced by data modeling is the normal variation of psychophysical measures of thresholds. The normal variability in threshold measurements, when the thresholds are determined by probability summation, is an especially important factor in the early detection of glaucoma. Because the relationship between the visual threshold and number of neural mechanisms is logarithmic, a relatively large proportion of ganglion cells (40%–50%) must be lost before the threshold measurement exceeds the normal variability and reaches statistical significance. Consequently, the sensitivity of perimetry for early detection should be higher in visual field locations with lower variability, but the slope of the structure–function relationship also varies with eccentricity and offsets the benefit of reduced variability. For this reason, the initial diagnosis of significant visual field defects with standard perimetry is associated with approximately equal proportions of ganglion cells death in all retinal locations. However, it may be possible to take advantage of reduced measurement variance with alternative methods of perimetry based on ganglion cell-specific stimuli, such as frequency-doubling technology or motion stimuli,[45–49] that reduce measurement variance at all test locations and/or reduce the number in the pool of potential stimulus detectors.

The use of alternative methods of perimetry stimuli, with ganglion cell-specific stimuli, can be more efficient than white-light stimuli for detecting the initial losses of ganglion cells,[46,47,50] but these stimuli may not be more effective in following the progression of established visual field defects. Once the neuropathy has progressed to the level of clinical significance, then there is a high correlation between different perimetry procedures that have been designed to selectively test very different ganglion cell populations.[37,50–58] It is likely, therefore, that the characteristics for structure–function relationships with alternative methods of perimetry will be very similar to those of standard clinical perimetry.

In summary, the present study has shown that neural losses from experimental glaucoma are well correlated with visual losses in standard clinical perimetry when eccentricity factors are included. The structure–function relationships at each eccentricity are linear on log–log coordinates and the parameters from linear regression vary systematically across eccentricity. The orderly behavior of each of the variables of the structure–function relationships provided the framework for a model to

predict the ganglion cell density underlying a given level of visual sensitivity and location in the visual field. The model's success in predicting retinal ganglion cell losses from both experimental glaucoma in monkeys and normal aging in humans suggests that it has potential for application for the clinical interpretation of the state of glaucomatous optic neuropathy. However, before the clinical application can be instituted, further development may be necessary using data from human glaucoma patients.

References

1. Anderson DR. *Perimetry, With and Without Automation,* 2nd ed. St. Louis: C.V. Mosby, Co; 1987.
2. Johnson CA. Standardizing the measurement of visual fields for clinical research. *Ophthalmology*. 1996;103:186–189.
3. Alexander LJ. Diagnosis and management of primary open-angle glaucoma. In: Classe JG, ed. *Optometry Clinics.* Norwalk, CT: Appleton & Lange; 1991:19–102.
4. Quigley HA. Open-angle glaucoma. *N Engl J Med*. 1993;328:1097–1106.
5. Epstein DL. Primary open angle glaucoma. In: Epstein DL, Allingham RR, Schuman JS, eds. *Chandler and Grant's Glaucoma.* 4th ed. Baltimore: Williams & Wilkins; 1997;183–198.
6. Johnson CA, Sample PA. Perimetry and visual field testing. In: Kaufman PK, Alm A, eds. *Adler's Physiology of the Eye*. St. Louis: CV Mosby Co; 2003;552–577.
7. Advanced Glaucoma Intervention Study. 2. Visual field test scoring and reliability. *Ophthalmology.*1994;101:1445–1455.
8. Kass MA, Heuer DK, Higginbotham EJ, et al. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch Ophthalmology*. 2002;120:701–713.
9. Heijl A, Leske MC, Bengtsson B, Hyman L, Bengtsson B, Hussein M; Early Manifest Glaucoma Trial Group. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. *Arch Ophthalmol.* 2002;120:1268–1279.
10. Quigley HA, Dunkelberger GR, Green WR. Retinal ganglion cell atrophy correlated with automated perimetry in human eyes with glaucoma. *Am J Ophthalmol*. 1989;107:453–464.
11. Harwerth RS, Carter-Dawson L, Shen F, Smith EL, Crawford MLJ. Ganglion cell losses underlying visual field defects from glaucoma. *Invest Ophthalmol Vis Sci*. 1999;40:2242–2250.
12. Kerrigan-Baumrind LA, Quigley HA, Pease ME, Kerrigan DF, Mitchell RS. Number of ganglion cells in glaucoma eyes compared with threshold visual field tests in the same persons. *Invest Ophthalmol Vis Sci*. 2000;41:741–748.
13. Harwerth RS, Crawford ML, Frishman LJ, Viswanathan S, Smith EL 3rd, Carter-Dawson L. Visual field defects and neural losses from experimental glaucoma. *Prog Retina Eye Res*. 2002;21:91–125.
14. Anderson DR, Knighton RW. Perimetry and acuity perimetry. In: Shields MB, Pollack IP, Kolker AE, eds. *Perspectives in Glaucoma.* Thorofare, NJ: Slack, Inc; 1988:59–70.
15. Garway-Heath DF, Caprioloi J, Fitzke FW, Hitchings RA. Scaling the hill of vision: the physiological relationship between light sensitivity and ganglion cell numbers. *Invest Ophthalmol Vis Sci*. 2000;41:1774–1782.
16. Swanson WH, Felius J, Pan F. Perimetric defects and ganglion cell damage: interpreting linear relations using a two-stage neural model. *Invest Ophthalmol Vis Sci*. 2004;45:466–472.
17. Pirenne MH. Binocular and monocular thresholds for vision. *Nature*. 1943;153:698–699.
18. Nachmias J. On the psychometric function for contrast detection. *Vision Res*. 1981;21:215–223.
19. Robson JG, Graham N. Probability summation and regional variation in contrast sensitivity across the visual field. *Vision Res*. 1981;21:409–418.
20. Tolhurst DJ, Movshon JA, Dean AM. The statistical reliability of signals in single neurons in cat and monkey visual cortex. *Vision Res*. 1983;23:775–785.
21. Harwerth RS, Smith EL. The intrinsic noise of contrast sensitivity perimetry. In: Wall M, Wild J, eds. *Perimetry Update 2000/2001.* The Hague, The Netherlands: Kugler Publications; 2001:59–68.
22. Drasdo N. Receptive field densities of the ganglion cells of the human retina. *Vision Res.* 1989;29:985–988.
23. Curcio CA, Allen KA. Topography of ganglion cells in human retina. *J Comp Neurol.* 1990;300:5–25.
24. Curcio CA, Drucker DN. Retinal ganglion cells in Alzheimer's disease and aging. *Ann Neurol.* 1993;33:248–257.
25. Rolls ET, Cowey A. Topography of the retina and striate cortex and its relationship to visual acuity in rhesus monkeys and squirrel monkeys. *Exp Brain Res.* 1970;10:298–310.
26. Perry VH, Cowey A. The ganglion cell and cone distributions in the monkey's retina: implications for central magnification factors. *Vision Res.* 1985;25:1797–1810.
27. Wassle H, Grunert U, Rohrenbeck J, Boycott BB. Cortical magnification factor and the ganglion cell density in the primate retina. *Nature.* 1989;341:643–646.
28. Wassle H, Grunert U, Rohrenbeck J, Boycott BB. Retinal ganglion cell density and cortical magnification factor in the primate. *Vision Res.* 1990;30:1897–1911.
29. Flammer J, Drance SM, Augustiny L, Funkhouser A. Quantification of glaucomatous visual field defects with automated perimetry. *Invest Ophthalmol Vis Sci*. 1985;26:176–181.
30. Heijl A, Lindgren G, Olsson J. Normal variability of static perimetric threshold values across the central visual field. *Arch Ophthalmol*. 1987;105:1544–1549.
31. Heijl A, Lindgren G, Olsson J. Perimetric threshold variability and age. *Arch Ophthalmol*. 1988;106:450–452.
32. Harwerth RS, Smith EL, DeSantis L. Behavioral perimetry in monkeys. *Invest Ophthalmol Vis Sci.* 1993;34:31–40.
33. Heijl A, Lindgren G, Olsson J. A package for the statistical analysis of visual fields. *Documenta Ophthalmologica*. 1987;49:153–168.
34. Heijl A, Lindgren G, Olsson J. Reliability parameters in computerized perimetry. *Documenta Ophthalmologica*. 1987;49:595–600.
35. Harwerth RS, Smith EL, DeSantis L. Experimental glaucoma: perimetric field defects and intraocular pressure. *J Glaucoma.* 1997;6:390–401.
36. Harwerth RS, Smith EL 3rd, Chandler M. Progressive visual field defects from experimental glaucoma: measurements with white and colored stimuli. *Optometry Vision Sci*. 1999;76:558–570.
37. Frishman LJ, Saszik S, Harwerth RS, et al. Effects of experimental glaucoma in macaques on the multifocal ERG. Multifocal ERG in laser-induced glaucoma. *Documenta Ophthalmologica.* 2000;100:231–251.
38. Crawford ML, Harwerth RS, Smith EL, Shen F, Carter-Dawson L. Glaucoma in primates: cytochrome oxidase reactivity in parvo- and magnocellular pathways. *Invest Ophthalmol Vis Sci.* 2000;41:1791–1802.
39. Crawford ML, Harwerth RS, Smith EL, Mills S, Ewing B. Experimental glaucoma in primates: changes in cytochrome oxidase blobs in V1 cortex. *Invest Ophthalmol Vis Sci.* 2001;42:358–364.
40. Carter-Dawson L, Crawford ML, Harwerth RS, et al. Vitreal glutamate concentration in monkeys with experimental glaucoma. *Invest Ophthalmol Vis Sci.* 2002;43:2633–2637.
41. Harwerth RS, Smith EL. Rhesus monkey as a model for normal vision of humans. *Am J Optometry Physiol Optics.* 1985;62:633–641.
42. Blanks JC, Torigoe Y, Hinton DR, Blanks RHI. Retinal pathology in Alzheimer's disease. I. Ganglion cell loss in the foveal/parafoveal retina. *Neurobiol Aging.* 1996;17:377–384.
43. Harman A, Abrahams B, Moore S, Hoskins R. Neuronal density in the human retinal ganglion cell layer from 16 to 77 years. *Anatom Rec.* 2000;260:124–131.
44. Johnson CA, Adams AJ, Lewis RA. Evidence for a neural basis of age-related visual field loss in normal observers. *Invest Ophthalmol Vis Sci.* 1989;30:2056–2064.
45. Lachenmayr BJ, Airaksinen PJ, Drance SM, Wijsman K. Correlation of retinal nerve-fiber-layer loss, changes at the optic nerve head and various psychophysical criteria in glaucoma. *Graefes Arch Clin Exp Ophthalmol.* 1991;229:133–138.

46. Johnson CA. The Glenn A. Fry Award Lecture: Early losses of visual function in glaucoma. *Optometry Vision Sci*. 1995;72:359–370.
47. Sample PA, Bosworth CF, Blumenthal EZ, Girkin C, Weinreb RN. Visual function-specific perimetry for indirect comparison of different cell populations in glaucoma. *Invest Ophthalmol Vis Sci*. 2000;41:1783–1790.
48. Cello KE, Nelson-Quigg JM, Johnson CA. Frequency doubling technology perimetry for detection of glaucomatous visual field loss. *Am J Ophthalmol*. 2000;129:314–322.
49. Stroux A, Korth M, Junemann A, et al. A statistical model for the evaluation of sensory tests in glaucoma, depending on optic disc damage. *Invest Ophthalmol Vis Sci*. 2003;44:2879–2884.
50. Johnson CA. Psychophysical measurement of glaucomatous damage. *Survey Ophthalmol*. 2001;45(suppl 3):S313–S318.
51. Sample PA, Madrid ME, Weinreb RN. Evidence for a variety of functional defects in glaucoma-suspect eyes. *J Glaucoma*. 1994; 3(suppl 1):S5–S18.
52. Lynch S, Johnson CA, Demirel S. Is early damage in glaucoma selective for a particular cell type or pathway? In: Wall M, Heiji A, eds. *Perimetry Update 1996/1997*. Amsterdam: Kugler Publications; 1997:253–261.
53. Sample PA. What does functional testing tell us about optic nerve damage? *Survey Ophthalmol*. 2001;45(suppl 3):S319–S324.
54. Ansari EA, Morgan JE, Snowden RJ. Glaucoma: squaring the psychophysics and neurobiology. *Br J Ophthalmol*. 2002;86:823–826.
55. Ansari EA, Morgan JE, Snowden RJ. Psychophysical characterization of early functional loss in glaucoma and ocular hypertension. *Br J Ophthalmol*. 2002;86:1131–1135.
56. Martin L, Wanger P, Vancea L, Gothlin B. Concordance of high-pass perimetry and frequency-doubling technology perimetry results in glaucoma: no support for selective ganglion cell damage. *J Glaucoma*. 2003;12:40–44.
57. Kadaboukhova L, Lindblom B. Frequency doubling technology and high-pass resolution perimetry in glaucoma and ocular hypertension. *Acta Ophthalmol Scand*. 2003;81:247–252.
58. Iester M, Altieri M, Vittone P, Calabria G, Zingirian M, Traverso CE. Detection of glaucomatous visual field defect by nonconventional perimetry. *Am J Ophthalmol*. 2003;135:35–39.