Visual field defects and neural losses from experimental glaucoma

Ronald S. Harwerth\textsuperscript{a,}\textsuperscript{*}, M.L.J. Crawford\textsuperscript{b}, Laura J. Frishman\textsuperscript{a}, Suresh Viswanathan\textsuperscript{a,1}, Earl L. Smith III\textsuperscript{a}, Louvenia Carter-Dawson\textsuperscript{b}

\textsuperscript{a}College of Optometry, University of Houston, 505 J. Davis Armistead Building, Houston, TX 77204-2020, USA
\textsuperscript{b}Department of Ophthalmology and Visual Science, University of Texas Medical School at Houston, Houston, TX 77030, USA

Abstract

Glaucoma is a relatively common disease in which the death of retinal ganglion cells causes a progressive loss of sight, often leading to blindness. Typically, the degree of a patient’s visual dysfunction is assessed by clinical perimetry, involving subjective measurements of light-sense thresholds across the visual field, but the relationship between visual and neural losses is inexact. Therefore, to better understand of the effects of glaucoma on the visual system, a series of investigations involving psychophysics, electrophysiology, anatomy, and histochemistry were conducted on experimental glaucoma in monkeys. The principal results of the studies showed that, (1) the depth of visual defects with standard clinical perimetry are predicted by a loss of probability summation among retinal detection mechanisms, (2) glaucomatous optic atrophy causes a non-selective reduction of metabolism of neurons in the afferent visual pathway, and (3) objective electrophysiological methods can be as sensitive as standard clinical perimetry in assessing the neural losses from glaucoma. These experimental findings from glaucoma in monkeys provide fundamental data that should be applicable to improving methods for assessing glaucomatous optic neuropathy in patients. © 2002 Elsevier Science Ltd. All rights reserved.
1. Introduction

Clinical glaucoma is usually described as a multifactorial disease or as a constellation of diseases because there is not an identifiable single etiology (Quigley, 1993; Epstein, 1997). It is well known, however, that there are epidemiological risk factors that are associated with an increased likelihood of having the disease (Quigley, 1993; Gramer and Tausch, 1995) and there are cellular-level risk factors leading to pathologic injury and death of retinal ganglion cells (Schumer and Podos, 1994; Nickells, 1996; Quigley, 1998a). In the final analysis, all of the etiological factors lead to a single manifestation of glaucoma, the death of retinal ganglion cells, which can be observed in patients by a cupping of the optic nerve head, a loss of retinal nerve fiber layer, and functional visual field defects (Anderson, 1989; Alexander, 1991). Consequently, clinical procedures for the diagnosis and assessment of the progression of glaucoma are based on quantification of these characteristics of optic neuropathy and, thus, it is important to know how well the diagnostic techniques provide a true representation of the extent of ganglion cell death. For this purpose, we have investigated the functional and structural effects of glaucomatous optic neuropathy in an experimental monkey-model of glaucoma. The present report will describe investigations that were designed to study: (1) the relationship between the depth of visual field defects and the loss of retinal ganglion cells from glaucoma, (2) the relative effects of glaucoma on neurons in the magnocellular and parvocellular afferent visual pathways, and (3) the use of the electroretinogram (ERG) for objective measurements of retinal neural losses from glaucoma.

2. Structure–function relationships for clinical perimetry

In modern clinics, the standard for assessment of functional vision defects is visual field testing by computer-automated perimetry, using white-light test targets superimposed on a white background (Heijl, 1985a; Johnson, 1996). In general, the depth and extent of functional defects are diagnosed by a sensitivity map of the visual field that is derived from light-sense thresholds measured at a number of locations across the retina (Anderson, 1987). The visual field defects from glaucoma are characterized by a progressive loss of sensitivity that typically begin in the mid-periphery of the nasal field and eventually extend to the central visual field (Drance, 1985; Heijl, 1985b; Mikelberg et al., 1986; Quigley, 1993). The progression of visual field defects in glaucoma is an indication of progressive pathological losses of retinal ganglion cells with the extent of neural damage related to the amount of increase in light-sense thresholds (Heijl et al., 1987a,b; Katz et al., 1991, 1997; Asman and Heijl, 1992).

The clinical interpretation of visual field defects is not straightforward because the structure–function relationship for glaucoma, i.e., the relationship between losses in visual sensitivity and retinal ganglion cells in eyes of subjects with glaucoma, has significant inaccuracy and imprecision (Quigley et al., 1989; Harwerth et al., 1999a; Kerrigan-Baumrind et al., 2000). First, there is an early inaccuracy because a certain amount of ganglion cell loss must occur before significant visual field defects can be detected by standard perimetry. Secondly, the relationship lacks precision because of the considerable variability in the sensitivity losses that are caused by any given amount of ganglion cell loss. Several hypotheses have been proposed for the lack of accuracy and precision in the structure–function relationship for standard clinical perimetry (Quigley et al., 1988a, b; Quigley, 1993; Frisen, 1993; Johnson, 1994) which have also motivated investigations of new perimetry techniques to improve the fundamental relationship (e.g., Frisen, 1993; Johnson et al., 1993; Wall and Ketoff, 1995; Johnson and Samuels, 1997; Sample et al., 1999).

Regardless of the perimetry technique, the structure–function relationship that underlies the subjective assessment of visual fields depends on two components: (1) well controlled psychophysical measurements of visual thresholds, and (2) a specific psycho-physiological link between the sensory and neural substrates. The ideal psycho-physiological link would be a direct linear relationship so that the level of sensitivity at any location on the retina is directly proportional to the number of viable cells in the corresponding retinal ganglion cell layer (Brubaker, 1996). However, as previous studies have shown, factors other than retinal ganglion cell loss affect the level of visual sensitivity (Wild et al., 1995; Wall et al., 1996; Henson et al., 2000; Bengtsson and Heijl, 2000; Harwerth and Smith, 2001), which may be related to psychophysical methodology or to the psycho-physiological linking. It is, therefore,
important to consider the effects of basic psychophysical principles on the measurement of visual thresholds from perimetry, as for example, the recent studies that have shown that the precision of perimetry thresholds is inversely related to depth of the visual defect from glaucoma (Heijl et al., 1989; Chauhan et al., 1993; Wall et al., 1997; Hensor et al., 2000) and others demonstrating that the level of sensitivity is a function of the patient's response criterion (Kutzko et al., 2000).

Psychophysical methodology for threshold measurements has an extensive history of investigation and its application in computer-automated perimetry is well developed (Johnson et al., 1992; Bengtsson and Heijl, 1999; Wild et al., 1999). In comparison, relatively little is known about the second component of the structure–function relationship for perimetry, i.e., the relationship between sensory and neural substrates for visual thresholds (Quigley, 1998a). The elemental relationship between sensory and neural processes has been expressed in two models that relate neuronal activity to perceptual judgments: (1) the lower envelope model and, (2) the pooling model (Parker and Newsome, 1998). The lower envelope model is based on a principle that visual thresholds are set by the detection mechanism that has the lowest threshold for the specific stimulus, without influence from any mechanisms with less sensitivity. In the strictest sense, the lower envelope model predicts that the psychophysical thresholds are determined by the responses of single neurons. The alternative model, the pooling model, is based on the concept that visual thresholds are determined by the combination of signals from several sensory mechanisms. The pool adds together the responses from a group of mechanisms and the strength of the pooled signal determines the psychophysical response. The differences in the models may be arbitrary, depending on the definitions of the neural contributions to a detection mechanism and the number of detection mechanisms that make a pool, but both of the models and the available empirical evidence suggest that thresholds for most visual judgments are determined by the responses of small ensembles of neurons (Barlow, 1972; Tolhurst et al., 1983; Britten et al., 1992; Prince et al., 2000).

In terms of the clinical application to visual field defects from glaucoma, the general concepts of neural-perceptual relationships may help to explain the lack of accuracy and precision of the structure–function relationship for standard clinical perimetry (Quigley et al., 1989; Harwerth et al., 1999a; Kerrigan-Baumrind et al., 2000). For example, the amount of ganglion cell death that occurs before significant visual field defect is well explained by the lower envelop model, because the threshold would not be elevated until all of the detection mechanisms have been affected. Similarly, the variance in sensitivity loss as a function of ganglion cell death could be a result of decreased probability summation among independent detection mechanisms with heterogeneous degrees of damage affecting their response properties.

The present report addresses these issues of the structure–function relationship for standard clinical perimetry in monkeys with experimental glaucoma. The effects of alternative perimetry stimuli and the basic relationships between these alternative stimuli have also been considered.

### 3. Experimental glaucoma

Gaasterland and Kupfer (1974) introduced the model of experimentally elevated intraocular pressure (IOP) in monkeys, which has become the model of choice for anatomical and physiological investigations of glaucoma. In current use, experimental glaucoma is produced by a unilateral elevation of intraocular pressure 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 (Pederson and Gaasterland, 1984; Quigley and Holman, 1987; Marx et al., 1988; Harwerth et al., 1997). Using multiple treatments, separated by several weeks, the full 360° of the trabecular meshwork is treated to achieve sustained elevated pressures that are generally greater than 40 mmHg.

The validity of this animal model for the clinical pathology of glaucoma has been demonstrated by the similarities between the optic neuropathies (Pederson and Gaasterland, 1984; Quigley et al., 1995; Dreyer et al., 1996; Nickells, 1996) and functional defects (Harwerth et al., 1997, 1999b; Frishman et al., 2000; Hare et al., 1999, 2001) caused by experimentally elevated pressures in macaque monkeys and natural glaucoma in patients. The experimental model, however, provides several technical advantages for investigations of the specific relationship between neural and visual sensitivity losses. For example, the normal variance of the data for neural or sensory losses among patients may be reduced for unilateral experimental glaucoma, which compares the treated and control eyes of a single subject. A further advantage for such studies is that retinal tissue can be collected from monkeys at various stages of visual field defects to determine the neurologic damage underlying mild-to-severe 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.

For the present studies, experimental glaucoma was induced by laser treatment of one of the eyes of rhesus monkeys (Macaca mulatta) that had been trained for behavioral perimetry in order to follow the progression of visual field defects (Harwerth et al., 1993a, b). 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.

4. Behavioral perimetry

Static threshold perimetry has become the clinical standard for the assessment of the visual effects of glaucoma (Johnson, 1996). The sophisticated methodology and statistical analyses of perimetry data that have been developed for human patients can be also applied to experimental glaucoma through behavioral training and testing of the monkey subjects (Harwerth et al., 1993a). For these measurements, a standard clinical instrument, the Humphrey Field Analyzer, was attached to a primate-testing cubicle and the monkeys were trained to fixate and perform the same sort of detection task used for clinical perimetry. After the training was completed, standard automated perimetry using the 24-2 test pattern and the full-threshold test strategy with the Size III, white stimulus was used to assess the onset and progression of visual field defects caused by experimental glaucoma.

The monkeys became highly competent perimetry subjects and produced visual field data that are identical to the visual fields of humans in every important way. An example of the normal visual field for a monkey subject is presented in Fig. 1A. These perimetry data were obtained just prior to a laser treatment of the trabecular meshwork that caused a sustained elevation of the monkey’s intraocular pressure. The monkey had undergone three previous laser treatments and these typical data show that: (1) the normal thresholds and derived global indices for monkeys are comparable to those expected for normal human patients, and (2) even multiple laser treatments prior to the induction of ocular hypertension do not affect the perimetry threshold measurements.

The functional effects of elevated intraocular pressure (30–45 mm Hg) are shown by the subsequent visual field plots and threshold data in Figs. 1B–E. The perimetry data were obtained at approximately 6-week intervals and illustrate the rapid progression of visual field defects with experimental glaucoma. However, at any point in time during the course of progression, the visual field defects seem typical of those that could be seen in human glaucoma patients and the data from the monkeys can be analyzed and interpreted in the same way as for clinical patients. In the present experiments, retinal tissue samples from monkeys with various stages of visual field defects were collected to study the relationship between the losses in visual sensitivity and the status of the ganglion cell population.

5. Retinal ganglion cell counts

Within a few days after the final visual field test, the monkeys were deeply anesthetized, their eyes were enucleated, and 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 from specific perimetry test sites were taken from comparable retinal locations in the control and treated eyes (a total of 132 samples from 12 monkeys). The retinal locations for tissue samples were determined by the usual conversion ratio of 1 mm retinal distance per 4’ of visual angle (Wassle et al., 1990). This ratio was verified for the central visual field by comparing the distance from fixation to the center of the blind spot in the visual field to the direct measurement of the distance from the fovea to the center of the optic nerve head (Shen, 1994) but the conversion factor may not be linear at greater eccentricities (Perry and Cowey, 1984). In preparation for counting the ganglion cells, the retinal tissue samples were embedded and, subsequently, sectioned (1 μm thickness, radial sections) and stained (0.5% cresyl violet). Examples of the histology preparations are presented in Fig. 2. The examples demonstrate that the morphology of the retinas of treated and control eyes of the monkeys were essentially identical, except for the reduced number of cells in the ganglion cell layer (Frishman et al., 1996b). The amount of ganglion cell loss was quantified by counting the neurons under light microscopy with 100 × magnification. All of the neurons were counted in a 1 μm segment from each of 10 sections separated by a minimum of 10μm. The cell densities of the ganglion cell layers were calculated utilizing Abercrombie’s (1946) method 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 IOP (Frishman et al., 1996). Therefore, any difference in cell density between treated and control eyes largely is a reflection of a loss of ganglion cells, quantified by the percent difference of the treated eye compared to the control eye. However, because displaced amacrine cells range from about 3% in central retina to 25% in the temporal mid-peripheral retina, the absolute percentage of ganglion cell loss is slightly higher than reported.

6. Sensitivity vs. neural losses from experimental glaucoma

The relationship for the reduction of visual sensitivity as a function of the losses of retinal ganglion cells, for clinical perimetry using the standard Goldmann III test stimulus on a 31.5 asb adapting background, is presented in Fig. 3A. Each of the data points represents the sensitivity loss (i.e., the difference, in decibel (dB) units, between the thresholds for the control and treated eyes at a given test field location) as a function of ganglion cell loss (i.e., the percentage difference in the ganglion cell densities for the control and treated eyes) at the retinal locations corresponding to the test field images. It is apparent that the sensitivity–neural data show the general trends that would be expected, i.e., small sensitivity losses are associated with small ganglion cell losses and large sensitivity losses with large ganglion cell losses, but it is also evident that there is considerable variance in the sensitivity losses caused by any specific amount of ganglion cell loss. In this and other respects, the major attributes of the data from experimental glaucoma in monkeys compare well to recent reports of visual function and ganglion cell losses in donated eyes of human glaucoma patients (Quigley et al., 1989; Kerrigan-Baumrind et al., 2000).

In order to emphasize the significant trends of the data, the effects of scatter were reduced by the simple data manipulation of averaging the sensitivity losses over fixed ranges of ganglion cell loss. The adapted plot of the data, presented in Fig. 3B, represents the means and standard deviations of the sensitivity losses in each of seven equal-width bins of ganglion cell losses. The data then were fitted by a two-line function, one zero-slope segment for sensitivity losses from smaller amounts of ganglion cell loss and a second segment with a positive slope for the range of larger ganglion cell losses. This form of the data allows effective visualization of three important characteristics of the structure–function relationships for standard automated perimetry.

The first characteristic, the linear segment with a positive slope, indicates that, on average, the standard clinical protocol (Goldmann III (0.43’) white test stimulus) is a valid assessment of neural losses for advanced visual field defects. For sensitivity losses greater than about 10 dB, the loss of visual sensitivity from experimental glaucoma was proportional to the loss of ganglion cells, with a rate of 0.4 dB loss of sensitivity per percent loss of retinal ganglion cells. However, it is also important to note that, although the
average sensitivity loss is proportional to the neural loss over the higher range of ganglion cell loss, the variability in the relationship is large. As shown by the error bars, the standard deviations of sensitivity loss with advanced ganglion cell loss are 7–8 dB, making evaluations of absolute losses in retinal neurons very difficult or, more importantly, making assessments of significant progression of glaucomatous neuropathy by standard perimetry very complicated. Although the variance for a given patient could be less than the grouped data, the data from the group of subjects indicate that differences in sensitivity values of 14–16 dB would be required to exceed to 95% confidence limits for progressive changes in visual field defects.

The second characteristic of the data regarding structure–function relationships for conventional perimetry (Fig. 3B) is that the visual sensitivity losses are not correlated with retinal ganglion cell losses until a substantial number of neurons have been lost. The uncorrelated losses of sensitivity and neurons are important both in respect to the early detection of glaucoma and in demonstrating a period of progressive neural loss that cannot be detected by standard clinical perimetry. Thus, the flat portion of the function shows that there is a neural reserve of about 50%. In other words, the retina may have twice as many ganglion cells as is necessary for maximum visual sensitivity. However, the data should not be taken at face value as a suggestion that glaucoma treatment is not necessary until half of the ganglion cells have been killed. In the first place, although it is not known whether the small spot of light used for clinical perimetry is the best measure of general visual function, it is known that the treatment of glaucoma is more effective at early stages (Quigley, 1993; Epstein, 1997). Secondly, differences in the sensitivity–neural relationships for early and advanced visual field defects may be a result of probabilistic mechanisms for visual thresholds, rather than a difference in the rate of progression of neural losses.

The third characteristic of the structure–function relationship presented in Fig. 3B is that there is a loss of 6–8 dB in visual sensitivity over the range of smaller losses of ganglion cells where sensitivity losses appear to be uncorrelated with neural loss. This level of sensitivity loss extends to even the lowest ganglion cell losses, indicating that statistically significant threshold elevations may occur even prior to ganglion cell loss. Thus, the statistical analyses of clinical perimetry data, which are based on empirical data from normal age-matched subjects, can provide early statistical evidence of glaucoma at a time where the visual defects are not proportional to the amount of ganglion cell death (Heijl, 1985b; Heijl et al., 1987a; Kerrigan-Baumrind et al., 2000). This finding may be associated with an alteration in retinal ganglion cell function prior to the pathological...
loss of the cell body and could be important for the initiation of glaucoma treatment.

The data, as presented in Figs. 3A and B, represent the most fundamental relationship for the clinical interpretation of standard automated perimetry data and, although another form of data analysis may change the form of the plotted data, it would not alter the basic structure–function relationship. On the other hand, other analyses may provide information about the threshold mechanisms of perimetry, especially with respect to understanding the basic non-linearity for the smaller magnitudes of sensitivity and ganglion cell losses (Bartz-Schmidt and Weber, 1993; Garway-Heath et al., 2000).

7. Probability summation and visual field defects

By any model of neuronal to perceptual events, the relation between a psychophysical measurement of sensitivity and the neural mechanisms responsible for the level of sensitivity must involve interactions among the detection mechanisms over time and space. The statistical description for detection of a stimulus that is imaged on a retinal area with multiple detectors is probability summation (Pirenne, 1943; Nachmias, 1981; Robson and Graham, 1981). Probability summation is a well-established principle of psychophysical theory that is based on an assumption that, if there are many mechanisms that could detect a stimulus, then the stimulus will be detected when any one of the detection mechanisms responds. Within this framework, the non-linear relationship between sensitivity and retinal ganglion cell losses in glaucoma may be explained partially by reduced probability summation among the neural detectors as the number of viable retinal ganglion cells decreases.

The basic principle of probability summation is expressed by the probability for the detection of a stimulus of fixed intensity by a population of homogeneous detection mechanisms according to the relationship:

\[ p = 1 - (1 - p_i)^n, \]

where \( p \) is the probability of overall detection, \( p_i \) is the probability of detection by each of the individual mechanisms, and \( n \) is the number of available detection mechanisms (Pirenne, 1943). Thus, the exponential relationship indicates that the probability of detection is affected less by the loss of a small number of mechanisms from a large population than by the loss of the same number of mechanisms from a smaller population.

In glaucoma, an increase in perimetry thresholds from the death of ganglion cells could occur from either a decrease in the number of detection mechanisms participating in probability summation, or from a reduction in the sensitivities of the detection mechanisms involved in probability summation. At a given stage of neural loss, the overall threshold as a function of the number and the threshold of available detection mechanisms is

\[ a_n = a n^{-\beta}, \]

where \( a_n \) is the overall threshold from probability summation among \( n \) homogeneous detectors that are characterized by intensity–response properties in the form of Weibull functions with thresholds of \( a \) and psychometric slopes of \( \beta \) (Robson and Graham, 1981; Nachmias, 1981; Tolhurst et al., 1983). For example, if the detection mechanisms have a slope \((\beta)=2\), then threshold will be increased by \(\sqrt{2}\) for each halving of the number of detectors during progressive glaucomatous neuropathy. Although neither the slopes nor the thresholds of the independent detection mechanisms are homogeneous across the retina, the loss of visual sensitivity from reduced probability summation should be linear for sensitivity losses versus cell losses on log–log coordinates. In fact, the relationship between sensitivity loss and ganglion cell loss, when both are in decibel units, is quite linear with approximately 1.3 dB loss of sensitivity per dB reduction in the population of ganglion cells (Fig. 3C). In comparison to the relationship for the sensitivity loss as a percentage loss of ganglion cells (Figs. 3A and B), linearity of the function was accomplished by compression of the data for ganglion cell losses of less than 50% into the first bin (0–3 dB) of ganglion cell loss of the dB-scale (Fig. 3C).

The data from these investigations show that the reduced sensitivity for the standard white-light stimulus used for standard clinical perimetry can be attributed to the reduced effect of probability summation as the number of ganglion cells is lost during glaucoma. However, the causes of reduced probability summation are not explicit from the analysis because it could occur from either a reduction of the sensitivities of individual neurons or in the number of receptive fields in the area of the stimulus (Frisen, 1993; Johnson, 1994; Felius et al., 1999; Henson et al., 2000; Garway-Heath et al., 2000; Swanson et al., 2000). The contributions of these effects may be investigated by spatial summation measures, which are generally assumed to represent summation within receptive fields contributing to the threshold response (Sloan, 1961; Robson and Graham, 1981; Felius et al., 1999; Harwerth and Smith, 2000).

8. Spatial summation for perimetry thresholds

Probability summation involves interactions among receptive fields and the predicted reduction of sensitivity is based on the reduced number of independent
detection mechanisms, rather than the specific retinal location or type of perimetry stimulus. In contrast, spatial summation within the individual receptive fields of detection mechanisms may be more dependent on these factors (Bartlett, 1966). The interdependence of stimulus size and intensity for visual thresholds, known as Ricco’s law, is in the form of

\[ aL = k_1, \text{ for } a < a_c \text{ or } L = k_2, \text{ for } a \geq a_c, \]

where \( k_1 \) is a constant of proportionality for a threshold response to a stimulus with an area of \( a \) and an intensity of \( L \). \( a_c \) is the critical area beyond which reciprocity between \( a \) and \( L \) does not hold and, for stimulus areas larger than \( a_c \), threshold responses either increase at a slower rate or have a constant intensity, \( k_2 \). Ricco’s law has been verified for both central and peripheral visual fields, with larger critical areas for peripheral than central stimulus locations (Bartlett, 1966; Wilson, 1970; Baumgargt, 1972). The range of stimulus sizes that are available in the standard Goldmann stimuli for computer-automated perimetry are in the range of expected area–intensity reciprocity, although the largest size (1.72° diameter) may exceed normal critical areas, even in the mid-peripheral visual field.

For perimetry on patients with normal vision, the changes in sensitivity from varying the stimulus area are predictable, but spatial summation with normal sensitivities does not necessarily predict how the sensitivities will change during glaucoma. On the one hand, if normal summation areas represent the spatial extent of the receptive fields of retinal ganglion cells, then spatial summation should not change over the time course of glaucoma and sensitivities with one size stimulus should be predicted by measurements with another stimulus size. However, if there are inhomogeneities in the states of ganglion cells or in the neural damage in a tested retinal area, then spatial summation may involve more than the receptive field sizes of ganglion cells (Felius et al., 1999; Harwerth and Smith, 2000; Swanson et al., 2000).

In this respect, perimetry with stimuli that are either smaller or larger than the standard Goldmann III (0.43°) stimulus may provide better information for the diagnosis or progression of glaucoma (Wilensky et al., 1986; Gilpin et al., 1990). Specifically, it has been suggested that the use of a Goldmann I (0.11°) stimulus may improve the sensitivity for early defects and the use of the larger Goldmann V (1.72°) stimulus may reduce measurement variability with more advanced defects (Wall et al., 1997; Yamada et al., 2001). Perimetry procedures involving variation of the size of the test stimulus have a historical precedence in manual perimetry where the size and intensity of the test stimuli were varied to plot a series of isopters (Anderson, 1987). With the Goldmann perimeter much of the diagnostic power was achieved by comparisons of isopters obtained with stimuli of different sizes and intensities. Similar strategies of visual field plotting have not been thoroughly investigated for automated perimetry, but alterations of test field size are simple modifications of the clinical procedure that could be implemented easily.

The general relationship between visual sensitivity and test field size for standard automated perimetry is illustrated in Figs. 4 and 5, which shows the pre-treatment data for two monkeys. The increase in sensitivity with increasing stimulus size, which is apparent in the gray-scale plots with a standard palette, is quantified by the sensitivity–size function presented in Fig. 6. The data for the function represent the means and standard deviations of the sensitivities across all of the test field locations of the HFA 24-2 full-threshold program, excluding two points near the blind spot. Each of the data points was derived from 50 visual fields from pre-treatment or control eye measurements taken from 10 monkeys (five fields each). This plot presents the typical form of increased average sensitivity with decreased variance of the measurements as the stimulus field size increased (Wall et al., 1996). The data are also representative of the relative change in sensitivity at any given location in the visual field, with the whole function being higher for test locations close to fixation, or lower for peripheral test locations.

The lines drawn through the data of Fig. 6 are based on the classical concept of reciprocity for spatial summation (Ricco’s law), which predicts that the product of the stimulus’ intensity and area will be constant when the stimulus area is smaller than a critical area and, then, thresholds will be a constant intensity for sizes larger than the critical area. The ascending line is the predicted size–intensity relationship of Ricco’s law, a unit-slope line passing through the mean sensitivity for the Goldmann III stimulus. The perimetry thresholds show perfect reciprocity between size and intensity for the smaller stimuli, but the larger stimulus exceeds the critical area for the size–intensity trade-off. A flat line has been drawn though the sensitivity data with a Goldmann V stimulus to emphasize incomplete summation, but probability summation effects may produce a slope greater than zero, as has been shown in other studies (Felius et al., 1999).

Examples of the field defects at measured with each of the three different Goldmann stimuli, are presented in Figs. 4 and 5. The two sets of examples represent different stages of visual field defects, one animal with a moderate stage of visual field loss by standard perimetry (Fig. 4) and another animal with advanced defects (Fig. 5). In both cases the gray-scale plots give the appearance that the perimetry defects are more advanced with smaller stimuli than with larger stimuli. However, the global perimetry indices, based on deviations from average normal control data for each stimulus size, indicate about the same degree of field
defects for the Goldmann I and III stimuli for the animal with moderate losses and for the Goldmann III and V stimuli for the animal with advanced visual field defects.

More quantitative studies of the effects of spatial summation in two subjects with visual field defects were conducted by the point-by-point correlations of threshold intensities with the Goldmann III stimulus versus either a Goldmann I (Figs. 7A and B) or V (Figs. 7C and D) stimulus. The thresholds with different sizes of perimetry stimuli were measured during a period of time when the visual fields with the standard Goldmann III stimulus were significantly abnormal. In some instances, the visual sensitivity was so low that they could not be measured with a smaller stimulus size, but could be measured with a larger stimulus size. For example, for the Goldmann I vs. III measurements (Figs. 7A and B), although the majority of the data fall along a diagonal for the plot, there was an obvious floor-effect when the monkeys’ thresholds with the Size I stimulus were beyond the intensity range of the perimeter, even though thresholds were still within the range of measurement with the Size III stimulus. Similarly, for the Goldmann V vs. III threshold measurements (Figs. 7C and D), a few of the data points represent thresholds that were outside the range of stimulus intensities available for the

Fig. 4. The effects of the size of the test field on the measurement of visual field defects from experimental glaucoma. Grayscale plots and the derived global indices are presented for test fields of three diameters (size I = 0.11°, size III = 0.43°, and size V = 1.7°) for the pre-treatment (left column) and post-treatment (right column) for an animal with moderate visual field defects by standard clinical perimetry.
Goldmann III stimulus. Thus, to avoid floor-effects, any data points that were less than 3 dB for either test field were excluded in the analysis.

Linear regression analysis indicated that the best fitting linear functions had slopes near 1, as would be predicted by Ricco’s Law and, therefore, the slopes of the fitted functions were constrained to 1, but the y-intercept was allowed to vary to produce the smallest sum of squared residuals. The final result was a function (correlation coefficients of 0.65–0.83 across animals) with an intercept close to the average difference between the normal thresholds for the two stimulus sizes (Fig. 6). Accordingly, on average the visual deficits from experimental glaucoma appear to demonstrate the same properties of spatial summation as the normal eyes. However, for some monkeys there are systematic deviations associated with more advanced field defects. The data for subject OHT-30, presented in Figs. 7B and D, show greater increases in sensitivity with increased stimulus area than would be predicted from simple spatial summation. For example, in the comparison of sensitivities with Goldmann I and III stimuli (Fig. 7B) a disproportionate number of data in the 15–30 dB range of thresholds with a Goldmann III stimulus fall below the fitted line, indicating that the increase in sensitivity with the larger stimulus area is greater than predicted by Ricco’s law. A similar extra increase in sensitivity is shown by the disproportionate number of data for the
Goldmann V stimulus are higher than predicted from spatial summation in the range from 5 to 20 dB for the Goldmann III stimulus (Fig. 7D).

An analogous finding of an abnormally large effect of increased stimulus size in areas of low sensitivity has been reported for patients with glaucoma or other retinal diseases (Felius et al., 1999, Swanson et al., 2000). In those studies, the mechanism proposed to account for the effect was heterogeneity in the disease-induced neural damage. For example, if the damage to the ganglion cell mosaic from glaucoma is heterogeneous, then smaller stimuli may fall on retinal areas where all of the cells have reduced sensitivity, while the larger stimuli also would include retinal areas with more normal sensitivity. Our findings are consistent with such a hypothesis because, although with heterogeneous damage it may be equally likely that the larger stimulus would extend to retinal areas of lower or higher sensitivity, the areas of lower sensitivity would not affect probability summation. Thus, spatial summation will be equal to or greater than predicted from normal control data. However, extra-large spatial summation was not as common for experimental glaucoma as for clinical glaucoma (Fellman et al., 1989; Felius et al., 1999). With experimental glaucoma, while some monkeys demonstrated super-summation, the data for most of the subjects were consistent with normal spatial summation. Therefore, the rapidly progressing neural damage caused by high intraocular pressures in experimental glaucoma may be more homogeneous than the neural damage in clinical glaucoma.

Regardless of the specific properties of spatial summation in glaucoma, the spatial summation effects

![Graph](image-url)
of standard clinical perimetry are not likely to provide improved methods to quantify the basic structure–function relationship of the disease. In fact, linear transforms provide reasonable conversions of the perimetry data obtained with one stimulus size to that obtained with another, which does not provide any additional information about the mechanisms underlying visual defects. Further, the conventional analysis of visual fields by perimetry indices (mean deviation (MD) and pattern standard deviation (PSD)) using statistics based on normal visual fields data with Goldmann I or V test stimuli did not demonstrate differences in the time course for the onset or progression of significant visual field defects caused by experimental glaucoma (Harwerth and Smith, 2000). Therefore, the clinical results that a Goldmann I stimulus improves the sensitivity for diagnosing early glaucoma and that perimetry with a Goldmann V stimulus reduces measurement variability in more advanced cases are not explained by differences in the fundamental threshold mechanisms of spatial summation or probability summation.

The results of the studies of spatial summation imply that losses in sensitivity caused by reduced populations of ganglion cells from glaucoma arises from a combination of elevated thresholds of the neurons contributing to the receptive fields and an eventual drop-out of receptive fields in an affected retinal area. While the effects of these two factors cannot be isolated by normal perimetry threshold measurements, the general results suggest that perimetry techniques could be improved by using stimuli with summation properties that differ from those for the detection of an increment of white light in a small area of the visual field (Pearson et al., 2001). Modification of the properties of probability and/or spatial summation to produce a more optimal structure–function relationship during glaucoma might be accomplished by either of two dominant theories, i.e., (1) using perimetry stimuli that are designed to be more specific for the mechanisms that are affected the earliest in the disease (Quigley, 1993; Quigley et al., 1988; Johnson, et al., 1993; Wall and Ketoff, 1995; Frisen, 1995; Sample et al., 1999; Cello et al., 2000) or, (2) using stimuli that are more specific for detection mechanisms with less redundancy than the population of mechanisms for white light stimuli (Johnson, 1994, 1995). Another approach, one that is not based on a specific psychophysiological linking hypothesis, is an empirical comparison of perimetry data with an alternative stimulus to the data obtained by conventional clinical perimetry. If the sensitivity data with the proposed and standard stimuli are highly correlated, as were the data with different Goldmann sizes, then it is unlikely that the alternative method yields a new type of diagnostic information. On the other hand, the finding of a poor correlation of perimetry data would be suggestive of a different sensitivity–neural relationship that could improve clinical perimetry. Therefore, a correlation study of this type was conducted for contrast sensitivity perimetry in experimental glaucoma.

9. Contrast sensitivity perimetry

Spatial contrast sensitivity functions provide an excellent description of the response properties of the visual system because the spatial response profiles of the receptive fields that are the most sensitive to a given stimulus will match the spatial frequency characteristics of the stimulus. Therefore, a stimulus that is composed of a narrow band of spatial frequencies and is restricted in its spatial size has many desirable properties for clinical perimetry (Atkin et al., 1979; Lundh and Gottvall, 1995; Harwerth and Smith, 1996). The detection thresholds for such a stimulus will be based on the responses of a specific subset of receptive fields in each retinal area tested, which could represent a smaller, more selective population of ganglion cells that respond to the standard white light stimulus.

Recently, contrast sensitivity methods have been implemented clinically as frequency-doubling perimetry and several reports have shown that the technique has a high degree of sensitivity and specificity for the detection of visual losses in patients that were diagnosed with glaucoma by standard clinical perimetry (Johnson and Samuels, 1997; Cello et al., 2000; Landers et al., 2000; Trible et al., 2000; Paczka et al., 2001). It has not been established, however, whether the structure–function relationships underlying visual field defects with frequency-doubling perimetry are different from those with standard clinical perimetry with Goldmann III white stimuli. Therefore, the present study was undertaken to determine the degree of correlation between thresholds by standard perimetry and a research form of contrast sensitivity perimetry. The measurements were obtained from monkeys with visual field defects caused by experimental glaucoma. In general, the methods of contrast sensitivity perimetry with monkeys were the same as for behavioral clinical perimetry, except that the detection stimuli were Gabor patches (Harwerth and Smith, 1996, 1997). The narrowband Gabor patch stimuli were composed of a 1 c/deg horizontal sine wave carrier windowed by a 2-D Gaussian spatial filter. For visual field measurements, the stimuli were presented for 250ms durations on a video monitor at test locations corresponding to coordinates of the HFA 24-2 Threshold Program over the central 30° (vertical) by 48° (horizontal) of the visual field. The perimetry thresholds (dB units of stimulus attenuation) by each procedure were used in pointwise comparisons of thresholds with standard clinical peri-
Two examples of the relationships between the contrast sensitivities for the Gabor and Goldmann III stimuli are presented in Fig. 8. The data from the two types of perimetry are poorly correlated in both cases and the sensitivity for the Gabor patch reaches a floor when the sensitivity for the white light increment is as large as 20 dB. In this respect, the Gabor patch demonstrates properties that are characteristic of a very small stimulus (cf., Figs. 5A and B to Figs. 6A and B), even though its spatial extent (1° per cycle) is more than twice as large as the Goldmann III stimulus (0.43°). The small effective size of the Gabor stimulus may be the result of its specificity to the receptive field properties of a relatively small population of retinal ganglion cells, but perimetry with the Gabor patch was not equivalent to simply reducing the size of the standard stimulus. For instance, the usual perimetry indices (Mean Deviation and Pattern Standard Deviation) derived for contrast sensitivity perimetry were more efficient than standard perimetry at demonstrating early visual field defects caused by experimental glaucoma (Harwerth and Smith, 1996). The procedures also differed in other ways. First, significant visual field defects with contrast sensitivity perimetry were generally more diffuse than with standard clinical perimetry, i.e., the MD was almost always more highly significant than the PSD and, second, the smaller dynamic range for grating contrast sensitivities limited the depth of visual field defects that could be measured with this stimulus.

It is not certain that the results from contrast sensitivity perimetry can be generalized to the clinical procedure of frequency-doubling perimetry, but the general principles are probably the same. The concept of matching the stimulus properties to the receptive field properties of a specific subpopulation of retinal ganglion cells is the same and not apt to be negated by the substantial differences in the spatial and temporal parameters of the stimuli for these studies and for frequency-doubling perimetry. In addition, previous studies have shown similar results for a stimulus with a large area of a low spatial–high temporal frequency grating that is used for frequency–doubling perimetry and a more localized grating pattern similar to that used in the present study (Johnson et al., 1999).

The results with contrast sensitivity procedures support the hypothesis that a perimetry stimulus that reduces the redundancy of detection mechanisms will improve the sensitivity to early glaucomatous defects (Johnson, 1994, 1995). In extension, it seems reasonable that the psycho-physiological linking between threshold and neural losses are different for the Gabor pattern and for the standard Goldmann III stimulus, but it remains to be determined whether the structure–function relationship is more exact for grating stimuli than spots of light. Nor do the findings address the issue of whether the neural damage from glaucoma is more selective to one class of neurons or neurons in one of the processing streams of the afferent neural pathways. These issues are best addressed by the histochemistry described in the next set of experiments.

### 10. Effects of experimental glaucoma in the afferent visual pathways

By the time patients complain of vision loss, a very high percentage of ganglion cells have already become non-functional or have died and, thus, the detection and assessment of glaucomatous visual dysfunction relies on clinical perimetry. In primary open angle glaucoma, the first appearance of a glaucomatous scotoma is typically in the mid-peripheral nasal field, which is the area of the retina served by the ganglion cells whose arching axons enter the dorsal and ventral aspects of the optic disk (Epstein, 1997; Quigley, 1998a). Although this area of the retina is served by a mixture of morphological and functional ganglion cell types, all entering the optic disk in a retinotopic manner, considerable attention has been
directed to the functional characteristics of the more sparse (<10%) but larger, parasol ganglion (P\textsubscript{s}) cells. To a large extent, the focus on the functional properties of these cells has been based on the finding that a selective loss of the larger retinal ganglion cells occurs in glaucoma. This finding suggested that the optimal tactic for early detection of glaucoma would be to design test stimuli with characteristics that are best suited for the receptive fields of the parasol ganglion cells (Quigley et al., 1987, 1988a, b; Glovinsky et al., 1991, 1993).

The parasol ganglion cells are one of the principal classes of retinal ganglion cells that are anatomically and physiologically distinct, but not physically separated in the retina. They are the retinal ganglion cells that constitute the major population of the magnocellular pathway (M-cells) of the afferent neural pathway to the visual cortex, while the more numerous (70%) medium to smaller midget ganglion (P\textsubscript{m}) cells make up the parallel parvocellular division (P-cells) and the small bistratified ganglion cells contribute the koniocellular division (K-cells) of the retino-geniculo-cortical pathway (Rodieck and Watanabe, 1993; Casagrande, 1994; Dacey, 1996). The ganglion cell types of the separate divisions transmit distinctively different types of functional visual information to the cortex (for recent reviews see Dacey, 1999; Frishman, 2001). It is, therefore, a sound structure–function strategy to design perimetry stimuli that match the receptive field properties of a ganglion cell type that may be affected the earliest or to the greatest extent in glaucoma (Quigley, 1993, 1998b; Johnson 1994, 1995; Weber et al., 1998; Sample et al., 1998). However, the medium-sized white stimuli used for standard perimetry do not possess properties that are unique to the receptive fields of the neurons in any of the functional streams, which has been suggested as the reason for the inaccuracy in the structure–function relationship for early neural defects from glaucoma (Holopigian et al., 1990; Sample et al., 1992; Johnson et al., 1993; Frisen, 1995; Cello et al., 2000). On the one hand, if glaucoma causes a selective loss of the larger M-cells, then the earliest glaucomatous vision loss should be revealed by perimetry using stimuli with properties optimized for these neurons, such as motion or flicker perception (Korth et al., 1989; Bullimore et al., 1993; Casson et al., 1993; Wall and Kettoff, 1995; Bossworth et al., 1997; Johnson and Samuels, 1997). On the other hand, if the death of ganglion cells in glaucoma is non-selective across cell types, then clinical tests do not need to be based on the specific properties of one functional pathway, but rather, the stimuli should be designed to reduce the effects of probability summation and spatial summation, yet still reflect the state of the entire population (Lynch et al., 1997). Thus, whether selective or non-selective ganglion cell loss occurs in glaucoma is an important distinction for development of methods for empirical clinical testing and in understanding the structure–function relationship underlying the progression of glaucoma.

The most direct determination of the effects of glaucoma on the M- vs. P-cell processing streams, diminished metabolic activity in the afferent visual pathway is also a significant component of glaucomatous visual dysfunction prior to the death of ganglion cells. It is an important consideration in the development of clinically identifiable glaucoma that the ganglion cells must fail in their faithful conveyance of information before they die. The separate stages in the progression of glaucoma, from dysfunction, death, and clearance of ganglion cells, are protracted and may extend over many years (Mikelberg et al., 1986; Smith et al., 1996; Viswanathan et al., 1997; Katz et al., 1997; Anderson et al., 2001). Therefore, the earliest change in the glaucomatous process must be a dysfunction in transmittance of visual information, as opposed to the death of ganglion cells. The time between dysfunction and death of the ganglion cell is not known, but part of the variance between the parametric measurements and subsequent ganglion cell counts (Fig. 3) may be attributable to a quiescent dysfunction, rather than actual ganglion cell death. The differentiation between cell loss and cell dysfunction is important, but the traditional functional measures by psychophysics and/or electrophysiology cannot differentiate between these effects. Therefore, we have studied the early changes in the metabolism of ganglion cells and the neural structures that are further downstream in the afferent visual pathway (Crawford et al., 2000, 2001).

11. Cytochrome oxidase and neuronal metabolism

The effects of experimental glaucoma in the afferent visual pathways were assessed by quantification of cytochrome oxidase activity to identify changes in the
energy metabolism required for neural activity. Information transmission in a neuron expends energy, which in the main, is derived from the reduction of adenosine triphosphate (ATP) within the membranes of the cell’s mitochondria. Cytochrome oxidase (CO) is an essential enzyme in this process and the concentration of CO within the mitochondria of a cell body or cell processes is tightly correlated with the electrical activity of the neuron. For example, it has been well documented that blocking retinal ganglion cell activity by intravitreal injection of the sodium channel blocker tetrodotoxin (TTX) causes a rapid reduction in CO concentration within the mitochondrial membranes in the ganglion cells, reflecting a lowered metabolic demand. In turn, the downstream targets of ganglion cells (LGN and visual cortex) show similar reductions in energy demand as the afferent inputs from ganglion cells to those targets also are reduced (Wong-Riley and Carroll, 1984; Wong-Riley et al., 1989a, b). With TTX blockade, or with enucleation, the outputs from all classes of ganglion cells are blocked, depriving all the recipient structures of afferent input, thereby, reducing their metabolic demand. In contrast, metabolic demand in glaucoma will vary, first it will vary across the visual field with the depth of visual field defects from the regional death of ganglion cells, and second, there will be variation with respect to the parallel afferent pathway sub-divisions, if there is a selectively greater rate of death of one functional class of ganglion cells. Accordingly, the death or dysfunction of the retinal ganglion cells will cause a differential level of afferent activation that should be reflected in the energy metabolism of neurons in the recipient laminae of the LGN and the ocular dominance columns of the inputs to layer 4C of the visual cortex (Crawford et al., 2000, 2001).

12. Cytochrome oxidase reactivity in experimental glaucoma—lateral geniculate nucleus

The alterations in metabolic activity in the lateral geniculate nucleus were investigated in monkeys with behaviorally measured visual field defects from unilateral experimental glaucoma (Harwerth et al., 1997, 1999a). The monkeys’ brains were perfused, in situ, with paraformaldehyde fixatives, and then removed, dissected, dehydrated and sectioned (35 μm sections). Frozen sections were processed according to the protocol of Wong-Riley (1979, 1989) to visualize cytochrome oxidase. The grayscale images of these brain sections were analyzed for the density of the CO reaction (COR) product within the LGN and the primary V1 cortex. The glaucomatous changes within the afferent visual system were determined by comparisons of the COR levels of the laminae innervated by the glaucomatous experimental eye to the COR levels of the adjacent laminae connected to the untreated normal eye (Crawford et al., 2001).

The normal metabolic activity and CO reaction in the LGN of a normal monkey (Fig. 9A) did not differ between adjacent layers, with only a slightly higher apparent density gradient from the upper, parvocellular layer 6 to the magnocellular layer 1 at the bottom of the nucleus. The outlined wedge and the circled areas (Fig. 9A) indicate the areas where the CO reaction was measured from serial sections from the front to the back of the LGN, extending along the representation of the horizontal meridian of the visual field (see Fig. 12). In comparison to the normal LGNs, the adjacent layers of the LGNs from monkeys with experimental glaucoma had markedly different levels of metabolic activity. An example of the effects of severe experimental glaucoma on the CO density are presented in Fig. 9B. The tissue is...
from the LGN that was ipsilateral to the treated eye, in which the layers 5, 3, and 2 were innervated by the hypertensive right eye (IOP of approximately 35 mmHg for about 1 yr). The levels of COR indicate that metabolic activity was significantly reduced in both P- and M-cell layers that were connected to the eye with visual field defects. Moreover, the losses in CO densities in the treated eye’s LGN layers were equivalent for the P- and M-cell laminae, suggesting that both of the divisions of the afferent pathway were affected equally (Crawford et al., 2000).

The results for advanced glaucoma are clear, but in these cases any differential effects between the metabolism of the M- vs. P-cell layers could have been reduced as more of the ganglion cells of all sizes died and, therefore, similar measurements must also be made for earlier stages of visual field defects. The earliest metabolic effects from ganglion cell damage should be observed in the contralateral LGNs of monkeys with mild stages of glaucoma because the temporal visual field is not affected in characteristic progression of visual field defects until the later stages of the disease. The initial visual field defect in experimental glaucoma, illustrated in Fig. 1B, typically appears in the nasal field about 20–30° peripheral to fixation and then proceeds to cross the vertical meridian into the temporal field. The retinal ganglion cells that correspond to the initial visual field defect are in the temporal retina that project to the ipsilateral LGN and, consequently, a reduction in metabolism appears first in these laminae (Crawford et al., 2000). The contralateral LGN, however, should demonstrate an earlier stage of metabolic effects because it represents a less-advanced defect in the visual field. Consequently, for subjects with mild field defects, the ganglion cells in the nasal retina should be at earlier stages of glaucomatous dysfunction, death, and clearance of the cell bodies. In the present experiments, the contralateral LGNs of monkeys with clearly discernable visual field defects and reductions of metabolic activity of the ipsilateral LGN were examined. When the change in COR was compared for corresponding retinal locations in the ipsilateral and contralateral locations, as shown in Fig. 10, there were no significant differences in the relative effects of experimental glaucoma between the M-cell and the P-cell layers, i.e., in the stages of mild visual field defects, the alterations of metabolic activity are essentially uniform across cell types.

Another characteristic of the progressive loss of visual sensitivity in experimental glaucoma in monkeys is that the sensitivity of central vision is less affected than in the peripheral visual field (see Fig. 1E). The analogous peripheral vs. central alterations in metabolic activity also can be observed in the CO staining patterns of topographically related LGN sections, which are presented in Fig. 11. The upper panel (Fig. 11A) shows COR in a section of the ipsilateral LGN representing the peripheral hemifield, while the lower panel (Fig. 11B) is a section with foveal representation in the same LGN. It is apparent that the ratio of COR for the control to treated eyes (layer 6/layer 5) is higher for the LGN layers related to the peripheral visual field than to those corresponding to the central visual field.

Metabolic alterations of the types illustrated by the individual histological sections in Figs. 9–11 were quantified by the gradients of metabolic effects across the visual field. The general agreement between the visual field and metabolic alterations is shown by data in Fig. 12. The monkey for these measurements had moderately advanced visual field defects of the treated eye, as shown by the grayscale plots in the upper panel (MD of approximately −8 dB). The functional visual deficits were paralleled by a reduction in metabolic activity, quantified by the ratios of COR for laminae...
innervated by the treated and untreated eyes. The lower-left panel (Fig. 12A) presents data from serial sections of the ipsilateral LGN along the representation of the horizontal retinal meridian, from central vision into the depth of the peripheral, nasal-field scotoma. The loss in COR for the fovea was about 3% and progressed almost linearly to a loss of about 12% in the deepest region of the scotoma. It is also important that, although the COR gradient corresponded to the field defect, the ratios for the P-cell and M-cell layers were nearly parallel throughout the entire range, suggesting that there is no significant difference in the effect of glaucoma on these two subdivisions of the LGN, regardless of the magnitude of the metabolic defect. A similar graph of the COR change in the contralateral LGN (Fig. 12B) shows a slightly smaller effect, but with the same general relationship, with respect to both the visual field defects and the uniform effects of glaucoma on the M- and P-cell layers throughout (Smith et al., 1993).

The COR gradient across the visual field for this monkey is generally related to the relative ganglion cell loss underlying the visual field loss. The ganglion cell loss in the central retina (i.e., the average and SD of the ganglion cell losses corresponding to the four perimetry test locations at 3° × 3° from fixation) was 15.04% ± 8.76%, which resulted in an 8.54 ± 1.56 dB loss of visual sensitivity. In comparison, the loss of ganglion cells in the retinal locations corresponding to the deep nasal field defects (i.e., the average and SD of the ganglion cell losses corresponding to four nasal field test locations that are 21° or 27° peripheral and 3° superior or inferior to the horizontal midline) was 73.87% ± 8.14%, resulting in a 15.06 ± 1.07 dB loss of visual sensitivity. Obviously, the amount of ganglion cell loss and extent of reduction in COR are causally related, but an exact correlation would not be expected because of the many other independent factors that influence thresholds and cell metabolism.

13. Cytochrome oxidase reactivity in experimental glaucoma — visual cortex

Additional evidence on the neural effects of experimental glaucoma and the relationship between visual function and metabolic defects along the parallel information processing streams can be gathered from investigations of CO reactivity in the input layer 4C of the primary visual cortex. The projections of the M- and P-cell pathways remain segregated into the visual cortex and terminate in separate sub-laminae of input layer 4C. The upper portion, layer 4Cα, receives the projection from the M-cell laminae of the LGN, while the lower portion, layer 4Cβ, receives input from the P-cell laminae. The relative change in metabolism caused by experimental glaucoma within these two sub-divisions is illustrated in Fig. 13. The bottom panel (Fig. 13A) is a section from the ipsilateral LGN of a monkey with severe glaucoma, showing a substantial reduction of COR in the recipient layers 5, 3, and 2. The drastic metabolic deficit is also readily apparent in the projection to layer 4C of primary visual cortex (Fig. 13B) in the coronal section through the primary visual cortex that shows several cycles of the characteristic ocular-dominance columns (ODCs). The alternating CO-rich (untreated — control eye) and CO-pale (treated — glaucoma eye) columns extend over the full thickness of the cortex, but the depth of modulation of COR is the most pronounced in layer 4C. Further, within the ODC innervated by the glaucomatous eye, there is a greater reduction of COR within the lower portion (layer 4Cβ) than in the upper portion (layer 4Cα). These differences
are easily observed in the upper panel (Fig. 13C) by an enlarged segment of layer 4C (the arrow indicates the boundary between the sub-laminae). The lower sub-lamina 4C\(_b\), with input from the P-cell layers of the LGN, is quite CO-pale compared to the upper sub-lamina 4C\(_a\); which receives input from the M-cell layers of the LGN. When the ratios of COR between the sub-lamina of ODCs innervated by the treated glaucoma eye were compared to the COR in the adjacent ODCs from the untreated control eye, a greater reduction in metabolism occurred in the P-cell stream of afferent input to the cortex. Indeed, when the reductions in COR were compared for a large number of monkeys with varying degrees of experimental glaucoma, a greater reduction was found in layer 4C\(_b\) in every case (Crawford et al., 2000).

Interestingly, during the course of these experiments it was noted that changes in COR could be detected in the 4C layers of the primary cortex before observable changes in the LGN. An example is presented in Fig. 14 by the comparison of COR in sections of the ipsilateral LGN (Fig. 14A) and visual cortex (Fig. 14B) from a monkey with mild visual field defects from experimental glaucoma of short duration. Although an inter-laminar change in COR is not detectable within the LGN, the differences in COR across ocular dominance columns are quite obvious in the tangential section of layer 4C of the visual cortex.

The investigation of neuronal metabolism at the higher stage of the afferent pathway confirmed the results of study at the LGN that glaucoma does not produce more deleterious effect on the constituent ganglion cells of the M-cell pathway, relative to the P-cell pathway. Rather, both pathways are affected, although there appears to be an amplification of metabolic defects, which suggests that the cortical neurons are affected at an earlier stage than those in the geniculate nucleus. In addition, that the metabolism of cortical neurons innervated by the P-cells are affected more severely than those in the M-cell pathway. These findings of glaucoma-induced metabolic defects past the fourth order neurons in the visual pathway also suggest that neurons in the superficial layers of the visual cortex similarly may be affected.

Beyond the input layers of the primary visual cortex, the CO-rich blobs of layers 2–3 have been shown to have a predominant input from the P-cell layer 4C\(_b\), with a direct minority input from the K-cells of the LGN and a
small number of inputs to the center of the blob from the M-cell layer 4C2 (Livingstone and Hubel, 1984; Lund, 1988; Casagrande, 1994; Yoshioda et al., 1996; Ding and Casagrande, 1998). Although, the identity of the differential effects of experimental glaucoma in M- and P-cell pathways becomes more blurred in the cortex, the findings at more peripheral processing sites suggest that experimental glaucoma might produce a substantial decrease of COR within the blobs, with inhomogeneous alterations of COR if the neural inputs have separate spatial distributions, e.g., the M-cell inputs are in the centers of the blobs (Edwards et al., 1995). However, although the effects of glaucoma on both the blob size and COR within the blob were substantial (a 50% reduction in the average size of the blob and a 9% reduction of COR density) the distribution of COR density across the blob was uniform (Crawford et al., 2001). Therefore, these results have extended the description of the pathophysiology of glaucoma along the afferent chain of anatomic sites from the eye to the superficial layers of primary visual cortex, with an increasing effect on overall metabolism, but without an indication of a selectively greater effect on neurons in either of the parallel processing streams.

The overall results of these investigations of the alterations on metabolic structure–function relationships in the LGN and visual cortex have shown qualitative agreement with the visual field defects measured by standard clinical perimetry. The agreement is not surprising because the concentration of CO varies with the metabolic demand of neural activity and, therefore, levels of CO activity should be proportional to the levels of neural activation from the retinal ganglion cells, to the LGN, and then to the visual cortex. Consequently, both the greater reduction in CO reaction in the part of the LGN with topographical representation of the peripheral, compared to central, visual field and the greater reduction of CO reaction in
areas with nasal, compared to temporal, visual field topographical representation are in direct agreement with perimetric threshold measures across the visual field. Although the reduced metabolic activity in the higher visual areas could have been caused by a dysfunction from injury preceding ganglion cell death, rather than from cell death, the reductions seem more likely to result from cell death because they are associated with sensitivity losses that are greater than 10 dB and, therefore, follow a significant loss of retinal ganglion cells (see Fig. 3). Additional experiments on animals with subliminal visual field defects by standard perimetry are needed to provide a conclusive answer. Nevertheless, within the sensitivity of the measurement, the alterations of metabolic activity were uniform across cell types, even at the earliest stages that were investigated. Therefore, the results of these studies of energy metabolism are in agreement with the recent morphological investigations that revealed equivalent degrees of cell shrinkage in all LGN laminae and those that have demonstrated that ganglion cell losses from glaucoma are not selective, at least not between the M- and P-cell pathways at the level of the LGN (Smith et al., 1993; Vickers et al., 1997; Morgan et al., 2000; Weber et al., 2000; Yucel et al., 2000). In contrast, the results at the next higher level of visual processing (V1) are suggestive that glaucoma has a differential effect with more significant metabolic changes at anatomic sites innervated by P-cell (layer 4Cβ) vs. M-cell (layer 4Cz) inputs, which was manifested as a 50% reduction in the sizes of the cytochrome oxidase blobs in the superficial layers of striate cortex.

At face value, the histochemistry of neural metabolism appears to provide evidence against the notion that the inaccuracy and imprecision of the structure–function relationship for standard perimetry (Fig. 3) are caused by the non-selective nature of the standard perimetry stimulus. Rather, the related results from the behavioral and cellular studies suggest that improved clinical tests could be designed to reduce the effects of probability summation and spatial summation, while maintaining representation of the health of the entire population of ganglion cells. Although the diverse methods of investigation have produced compatible results, it is important that metabolic structure–function relationships are based on fundamentally different principles than the psychophysical structure–function relationships. The density of COR product will be determined by the average concentration of cytochrome oxidase at a given anatomic site of analysis, while according to psychophysics theory, visual thresholds for stimulus locations that are topographically represented at the anatomic site are determined by a small ensemble of neurons having the highest sensitivities. Obviously, the same process of ganglion cell death causes both the behavioral and cellular deficits, but the correlation of behavioral events and the extent of cellular changes will not necessarily be precise.

14. Electroretinographic measures of neural loss from glaucoma

Although subjective measures of visual function seem to be a direct, valid assessment of the clinical stage of visual impairment caused by glaucoma, there are degrees of inaccuracy and imprecision in the underlying structure–function relationship for standard automated perimetry that limit the interpretation of stage of neural loss. Alternative approaches with objective measures of glaucomatous neuropathy that do not rely on psychophysiological linking have been developed in recent years. One approach has involved the direct measurement of morphological changes caused by the loss of the axons of retinal ganglion cells at the optic nerve head or in the nerve fiber layer (Weinreb et al., 1998; Trible et al., 1999; Blumenthal et al., 2000; Hoh et al., 2000; Kremmer et al., 2000; Wollstein et al., 2000; Blumenthal and Weinreb, 2001). Another theoretically valid approach involves the direct measure of glaucomatous neuropathy from the reduced electrical activity generated by the retinal ganglion cell bodies or axons in the electroretinogram (ERG) (Frishman et al., 1996b; Viswanathan and Frishman, 1997; Graham and Kistner, 1998; Sutter and Bearse, 1999; Hood, 2000).

The ERG is an important clinical tool for assessing the functional integrity of the retina, in vivo, because the electrical response to a change in illumination can be recorded non-invasively, but the ERG in glaucoma requires the isolation of components that are specifically ganglion cell responses. While several procedures have been suggested, it is uncertain which will provide the most reliable reflection of the stage of glaucomatous loss of retinal ganglion cells. In order to study this form of the structure–function relationship, several ERG techniques for assessing retinal ganglion cell function have been investigated. The specific studies to be described have involved measurements of dark-adapted (scotopic) (Frishman et al., 1996a,b) and light-adapted (photopic) (Viswanathan et al., 1999a,b) full-field flash ERGs, pattern ERGs (Viswanathan et al., 2000) and multifocal ERGs (Frishman et al., 2000). Most of the ERG studies were conducted in monkeys whose visual fields were being monitored closely by static threshold perimetry, and in some cases, there were sufficient measures over time to relate progression by ERG outcomes and visual field global indices. Additional studies of photopic flash ERGs and visual field indices in patients with primary open angle glaucoma were conducted to establish the clinical merit of the results from experimental glaucoma (Viswanathan et al., 2001).
The general methods for the studies have been described previously (Frishman et al., 1996a; Viswanathan et al., 1999a, b, 2000). With the monkeys under ketamine and xylazine anesthesia, ERGs were recorded differentially between DTL fiber electrodes centered on the cornea of each of the eyes (Dawson et al., 1979). The ERG signals were amplified, filtered (DC—300 Hz), and digitized at 1 kHz with a resolution of 1 μV. Response averages were based on 5–10 trials for intense flashes, but for weaker stimuli, responses were averaged over 20–160 stimulus presentations delivered in 20 trial blocks. The averaged ERG data were smoothed by digital filtering of the largest Fourier component close to 60 Hz and repeated three-point weighted smoothing (0.25, 0.5, 0.25) to eliminate noise at frequencies >250 Hz.

Full-field stimuli were produced by rear illumination of a section of a white diffusing surface positioned very close to one eye (i.e., a Ganzfeld; 35 mm in diameter). The monkey’s non-tested eye was covered with a black cloth. The visual stimuli were light increments, either from darkness or superimposed on steady adapting backgrounds. The ERG stimuli were produced by light emitting diodes (LEDs). The stimuli were green light (peak output at 560 nm) in dark-adapted studies or for the steady green (560 nm) or blue (450 nm) backgrounds. The ERG stimuli were produced by light from proximal to bipolar cells (Sieving et al., 1988; Wakahayashi et al., 1988; Frishman and Steinberg, 1989a, b; Naarendorp and Sieving, 1991; Sieving and Wakahayashi, 1991). The STR is generated by stimulus intensities that are too weak to elicit a normal b-wave and the initial measurable response occurs at intensities near the absolute psychophysical thresholds of human observers (Sieving and Nino, 1988; Frishman et al., 1996a). The normal waveforms of STRs (illustrated in the left-hand column of Fig. 15C for a macaque’s eye) are characterized by a slow, negative-going potential that reaches its greatest amplitude in about 200 ms with an amplitude that is proportional to the stimulus intensity of the brief flashes (shown by the ascending series of responses).

The normal STR was greatly reduced or abolished by experimental glaucoma that had progressed to stages of substantial visual field loss (Frishman et al., 1996). The effects of experimental glaucoma are demonstrated in Fig. 15C by a comparison of the ERGs from the experimental eye (right column) and control eye (left column) of an animal with advanced glaucomatous visual field defects (MD = −31.5 dB). In this case, the STR for the experimental eye was essentially eliminated and, instead of the normal negative response, the entire ERG response was a positive waveform. The glaucomatous loss of retinal ganglion cells was specific to the STR without alteration of other components of the ERG, and the a- and b-waves of the control and experimental eyes were very similar (see Fig. 15B).

The degree of effect on the STR was qualitatively correlated with the stage of glaucomatous visual field defect, with smaller reductions in the STR associated with less severe defects, but the study did not include a test’s utility in studies of glaucoma. Instead, most of the previous studies of glaucomatous eyes in humans (e.g., Korth, 1997; Graham and Klistorner, 1998) or in monkeys (Marx et al., 1986; Johnson et al., 1989) have used photopic patterned stimuli to elicit a pattern ERG, a response that originates largely from retinal ganglion cells (Maffei and Fiorentini, 1981; Maffei et al., 1985). However, more current work has shown that there are specific components of the scotopic flash ERG that also originate from the inner layers of the retina and which potentially could provide objective measures of ganglion cell function. The scotopic ERG in experimental glaucoma is of particular interest because, in clinical glaucoma, it has been reported that there are scotopic sensitivity losses before visual field defects can be documented (Glovinsky et al., 1992) and the scotopic PII (b-wave), and to a lesser extent the scotopic threshold ERG response, are reduced (Korth et al., 1994).

15. Effects of experimental glaucoma on the scotopic flash ERG (STR)

The full-field flash ERG has been used widely to assess the function of photoreceptors and bipolar cells. The standard scotopic ERG response to brief intense flashes has an initial, negative a-wave that originates primarily from rod photoreceptors (Penn and Hagins, 1969), which is followed by the prominent positive b-wave that originates from rod-driven (On) bipolar cells with some contribution from the Müller (glial) cells (Miller and Dowling, 1970; Newman and Odette, 1984; Stockton and Slaughter, 1989; Gurevich and Slaughter, 1993; Xu and Karwoski, 1994; Robson and Frishman, 1995, 1999). Examples of the standard ERG waveform for the control and laser-treated (experimental) eyes of an animal with deep visual field defects (Fig. 15A) are presented in Fig. 15B.

Until recently it was believed that retinal ganglion cell activity is not detectable in the flash ERG, limiting the...
sufficient number of animals with mild field losses to formally correlate the changes in STR with perimetric sensitivity losses (Frishman et al., 1996b). The study was not extended to more monkeys because, although the investigations demonstrated the STRs potential for an objective quantification of glaucomatous optic atrophy, in practice the procedure is likely to have limited clinical application. The primary problem with the STR as a clinical procedure is the lengthy time for administration, specifically, the successful recording of STRs requires the patient to be completely dark-adapted (>30 min) and the very small responses (<10 μV) dictate extensive averaging to improve signal-to-noise ratios. Therefore, another series of experiments involved photopic ERG methods that produce larger signals and do not require dark adaptation and, thereby, should improve the utility of objective ERG measurements of retinal ganglion cell losses from glaucoma.

16. Effects of experimental glaucoma on the photopic flash ERG (PhNR)

The photopic ERG is a complicated response, which probably represents contributions from each class of neurons in the retina. For example, fully photopic ERGs that are elicited by full-field red (630 nm) stimuli superimposed on a rod-saturating blue (450 nm) background, are composed of the standard a-waves, b-waves, and d-waves, and additional slow negative potentials, the photopic negative response, that are not ordinarily recorded in clinical ERGs. The cellular origins of the standard ERG components are neurons that are not affected by glaucoma; the a-wave arises from cone photoreceptors and off-bipolar cells (Bush and Sieving, 1994; Sieving et al., 1994; Viswanathan et al., 1999a) and the b-wave arises from on-bipolar cells with a response truncation from other post-receptor cells (Bush and Sieving, 1994). However, in contrast to these well-known ERG waves, the later negative potentials reflect the pathophysiology of glaucoma (Viswanathan et al., 1999b). These negative potentials, descriptively named the photopic negative response (PhNR), originate in the spiking neurons of the inner retina and are essentially eliminated by administration of TTX or by advanced experimental glaucoma (Viswanathan and Frishman, 1997; Viswanathan et al., 1999b, 2000).

Examples of the normal PhNR and the effects of glaucoma are presented in Fig. 16 for an animal with advanced visual field defects caused by experimental glaucoma.
Fig. 16. Photopic ERG responses (PhNR) in experimental glaucoma. (A) Grayscale plots of the perimetry data of a monkey (OHT-17) with moderate visual field defects (MD = −12.6) of the experimental eye. Examples of the PhNRs to three stimulus intensities are presented for long (B) or brief (C) red flashes for the monkey’s control (left) and experimental (middle) eyes. The right column presents the control—experimental eye differences in the ERG waveforms at each stimulus intensity to illustrate the components that were eliminated by the pathophysiology of experimental glaucoma.

(visual field plots obtained at the time of the recordings are presented in Fig. 16A). The left columns present photopic ERGs from the monkey’s control eye for a range of stimulus intensities that were presented as flashes of long (200 ms—Fig. 16B) or brief (<5 ms—Fig. 16C) duration. The normal PhNR is characterized by a negative response following the b-wave (marked by the double arrows) that is markedly attenuated by the pathophysiology of experimental glaucoma. For example, ERG waveforms for the experimental eye with relatively advanced visual field defects (MD = 12.65 dB) did not exhibit the type of negative potentials (middle column of Figs. 16B and C) that were predominant in the PhNR from the control eye. The specific effects of glaucoma are more explicit in the records in the right-hand column that were derived from the differences between the ERGs for the control and experimental eyes to reveal the components that were most affected by experimental glaucoma. This method of determining the effects of experimental glaucoma, i.e., the difference between records from the control and experimental eyes of the same animal, is justified because the ERGs from the two eyes before laser treatment were always very similar, even though
they often differ between animals (Viswanathan et al., 2000). These data demonstrate that the PhNR that was eliminated by glaucoma was a slow, negative-going potential that was initiated by light onset and, as is more pronounced with the longer duration stimulus, also at light offset.

The examples in Fig. 16 demonstrate that the PhNR is reduced at a time when visual field defects are advanced and it is probable that similar structure–function relationships underlie both the electrophysiological and psychophysical measurements with advanced defects, i.e., when the losses of visual sensitivity are proportional to the ganglion cell losses from experimental glaucoma (see Fig. 3B). However, the PhNR is recorded using a full-field (Ganzfeld) stimulus and cannot be related to the pointwise measurements of visual sensitivity in perimetry. Thus, to compare the depth of visual field defects to the degree of reduction in the PhNR it is more appropriate to use the MD perimetry index, i.e., the weighted average deviation from normal sensitivity across all of the test field locations.

The comparison of objective and subjective measurements of the effects of experimental glaucoma from 14 monkeys are presented in Fig. 17A. The data represent the reduction of the PhNR of the experimental eye relative to the control eye (normalized to the control eye), as a function of the MD of the perimetric field of the experimental eye. Across animals, the PhNRs of the experimental eyes were significantly reduced with respect to the control eye when visual field measurements showed mild to moderate MD losses and, thereafter, further reductions of PhNR with field loss were quite gradual. The relationship between PhNR and MD appears to be linear, with a shallow slope and a non-zero y-intercept and, consequently, the group data predict that a significant reduction of PhNR amplitude precedes clinically significant MD defects, but progression may be more difficult to quantify.

The relationships between electrophysiological and psychophysical measurements have clear implications for the clinical glaucoma, but it must be determined whether these relationships from the group data also pertain to individual subjects. The issues of detection and progression of glaucomatous neuropathy were addressed by comparisons of ERGs and visual fields over the time-course of experimental glaucoma. The data for the PhNR amplitude as a function of the MD defect from visual fields are presented in Fig. 17B for two monkeys. When the data following laser-induced intraocular hypertension (solid symbols) are compared to the means and 95% confidence limits for normal values of each of the measurements (open symbols) it is apparent that PhNR amplitudes fall below the normal confidence limits when the visual field defects are still within a normal range. With deeper visual field defects, the reductions in PhNR amplitudes as a function of MD appear linear, but with a relatively low rate of change. The fitted line was based only on the post-treatment data that were outside the 95% confidence limits for normal PhNR amplitudes, in accordance with a concept that there is a discontinuity between early and late effects in the electrophysiological vs. psychophysical relationship, just as there was between the psychophysical vs. neural relationship presented in Fig. 3. Overall, these preliminary data from experimental glaucoma suggest that significantly reduced amplitudes of the PhNR occur early in the disease process and in later stages the further reductions in amplitude occur at a constant rate proportional to the perimetric MD defect. In addition, the experiments on progression in individuals show that the changes can be monitored by absolute PhNR amplitudes from experimental eyes (Fig. 17B), as well as, the relative change of the experimental with respect to the control eye (Fig. 17A).

17. The PhNR in humans with primary open angle glaucoma

Laser-induced experimental glaucoma in macaque monkeys provides a valid model of the cellular and functional effects of ganglion cell death in glaucoma, but differences in the levels of intraocular pressure and in the time course of neural loss may affect the clinical application of some structure–function relationships (Osborne et al., 1999). It is, therefore, important to determine whether the experimental measures can be replicated in a clinical population of glaucoma patients (Colotto et al., 2000; Drasdo et al., 2001; Viswanathan et al., 2001).

A clinical investigation of PhNR vs. MD was conducted on 62 normal subjects (16–82 yr of age), 18 patients with primary open-angle glaucoma (POAG; 47–83 yr of age) and 7 POAG suspects (46–73 yr of age) (Viswanathan et al., 2001). Inclusion criteria for patients with POAG were a subset of the criteria used in the Collaborative Initial Glaucoma Treatment Study (Musch et al., 1999). All of the POAG patients met the following criteria: (1) an intraocular pressure (IOP) of ≥21 mmHg on at least two occasions prior to treatment, (2) an optic nerve head cup to disc ratio (C/D) of ≥0.6, and (3) reproducible visual field defects on the Humphrey 24-2 threshold test that included at least two contiguous points in the same hemifield on the total deviation probability plot at the <2% level. Patients with ocular disease other than POAG were excluded and the IOPs of most patients were controlled medically at the time of the study. All of the POAG suspects had a history of elevated IOP, but only satisfied one of the other inclusion criteria.
The experimental methods with human subjects were generally the same as was used for monkeys; DTL electrodes to record the PhNRs to brief (<6 ms), red, full-field Ganzfeld flashes superimposed on a steady, rod-saturating, blue background. PhNRs were clearly identifiable in the photopic ERGs of all subjects with normal eyes, but they were reduced or absent for the POAG patients and for most of the POAG suspects. The phenomenological differences in the PhNRs from normal and glaucomatous eyes are shown in Fig. 18A. The two sets of ERG records represent the PhNR of a typical normal subject on the left and for a POAG patient of similar age, but with a severe loss in visual sensitivity (MD = −16.2 dB) on the right. Importantly, although PhNRs were absent in the patient’s ERG, the a- and b-waves were similar to those of the normal subject, as was true for the entire sample of patients and age-matched controls.

The relationships between the PhNR amplitude and the visual field MD for each of the POAG patients are presented in Fig. 18B. Without a normal control eye for comparison as in unilateral experimental glaucoma, the PhNRs of the patients were taken from the absolute amplitude, which on average was about 20 μV for the group of normal subjects. Across the population of POAG patients, the PhNRs were reduced even when visual sensitivity losses were small and with larger degrees of sensitivity loss the amplitude of PhNR was

![Fig. 17. ERG amplitudes as a function of the depth of visual field defects in monkeys with experimental glaucoma. (A) The normalized differences between the control and experimental eyes of the PhNR amplitudes to brief stimuli as a function of the MD of the perimeter data. Each symbol represents the data for an individual subject. The mean difference between eyes and the 95% confidence limits of the pre-treatment data are shown by the solid and dashed lines. (B) The PhNR amplitudes (stimulus intensity of 1.7 log phot t.d.s⁻¹) for the two animals (OHT-30: squares) and OHT-28: circles) as a function of the MD of the perimeter data. The open symbols represent the pre-treatment data, with error bars denoting the 95% confidence limits for the monkey’s normal PhNR (vertical error bar) and MD (horizontal error bar), and the solid symbols represent data obtained at various times following laser-treatment to create experimental glaucoma. The line superimposed on the data was obtained by linear regression of the data outside of the 95% confidence limits for the normal PhNR data. (C) The PIN2 amplitudes of the mERGs (average data for stimulus rings 1–3 shown in Fig. 20) for the same subjects and recording sessions as the PhNR data in panel B. Details of the figure are the same as for panel B, except that the superimposed line was obtained by linear regression of the data outside of the 95% confidence limits for the normal visual field (MD) data.](image-url)
correlated significantly \( (p < 0.05) \) with MD. Thus, in all respects, the photopic ERGs in patients with POAG and in age-matched controls produced similar results to those found in treated and control eyes of monkeys with experimental glaucoma. This finding is important with respect to the structure–function relationships between PhNR amplitude and ganglion cell function because it supports the concept that the PhNR is determined by the number of normal retinal ganglion cells, rather than some of the other multifactorial conditions that are associated risk factors for clinical glaucoma.

The PhNR of the flash ERG and the effects of glaucoma only recently have been described (Viswanathan et al., 1999b, 2001; Colotto et al., 2000; Drasdo et al., 2001) but it is quite likely that the PhNR will be at least as sensitive as the pattern ERG (PERG) as an objective method for detecting glaucomatous damage. The PERG and particularly the slow negative potential, \( N_{95} \), that peaks about 95 ms after each contrast reversal in the transient PERG, has been shown in numerous studies to be altered in glaucomatous eyes (Thompson and Drasdo, 1987; Hess and Baker, 1984; Holder, 1987) and to be a sensitive indicator of glaucoma (e.g., Graham et al., 1996). Comparisons of the PhNR of the uniform field ERG and the transient PERG in monkeys with experimental glaucoma or with intravitreal injections of TTX indicated a common origin of the PhNR and PERG (Viswanathan et al., 2000). The sensitivity and specificity of the PhNR for detecting glaucoma in both humans and monkeys compares favorably with similar analyses in PERG measurements in humans patients (Graham et al., 1996). In addition, the PhNR elicited with red stimuli on a blue background is larger than the pattern ERG in the same animal, at least by a factor of two and large signals are easier to measure (Viswanathan et al., 2000). Other important advantages of the PhNR over the PERG are that it is less affected by opacities in the ocular media and it does not require correction of refractive errors.

18. The effect of experimental glaucoma on the multifocal ERG

A limitation of the use of full field stimuli for flash ERGs or large fields for pattern ERGs is that these stimuli do not allow measurements of localized functional losses that are the hallmark of perimetric measurements (Anderson, 1987; Quigley, 1993). One alternative would be to record focal ERGs, but the amplitudes of responses to focal stimuli are small and the testing of multiple sites would be very time consuming. A potentially more effective procedure is the multifocal (mf) ERG that has been recently developed (Sutter, 1991; Sutter and Tran, 1992) which produces simultaneous recordings of focal responses from more than 100 different retinal regions. The mfERG, therefore, provides the possibility for objective testing of localized retinal function. However, for studies of effects of glaucoma the extent to which retinal ganglion cell activity contributes to the mfERG responses must be determined.

An algorithm to extract a component generated at the optic nerve head by ganglion cell axons has been designed (Sutter and Bearse, 1999) which is based on the distinguishing feature of an optic nerve head component (ONHC), the change in latency of the waveform as a function of the distance from the optic nerve head. These changes create nasotemporal variations in local mfERG responses that are also unusually prominent in the ERG of the species of monkey (Macaca mulatta) used as subjects for experimental glaucoma (Hood et al., 1999a,b, 2001). The ONHCs are TTX-sensitive and can be eliminated by intravitreal injection of TTX or by a combination of TTX and NMDA (a glutamate agonist that further reduces
responses from retinal ganglion and amacrine cells) (Robson and Frishman, 1995; Hood et al., 1999a; Frishman et al., 2000).

The use of mfERGs as an objective method of assessing neural losses was investigated in monkeys with experimental glaucoma during the progression of their visual field defects (Frisman et al., 2000). The ERG stimuli consisted of 103 equal-sized hexagons; each about 3.3° wide, displayed on a white monochrome monitor with a video frame frequency of 75 Hz, but the actual duration of the light increment producing a hexagon was <1 ms. Commercial VERIS software was used to compute 1st and 2nd order kernels for the focal ERG from each hexagon and for pooled responses from groups of stimuli (Sutter, 1991; Sutter and Tran, 1992; Bearse and Sutter, 1996). The 1st order response in a linear system would be the impulse response of the retina at the location of each hexagon, but non-linearities, mainly from inner retina, are also present in the response. The 2nd order response is a non-linear response showing the effect of adaptation to successive flashes. In the present study, the first slice of the 2nd order response was measured, which incorporates the effect of the immediately preceding flash on the response (Sutter and Tran, 1992; Hood, 2000).

The normal nasotemporal variations in the mfERG were reduced in eyes with visual sensitivity losses from experimental glaucoma and were eliminated when those losses were profound. In order to illustrate the nasotemporal variation of the mfERG, data from hexagons were pooled by quadrants according to the stimulus pattern illustrated in Fig. 19. The recordings represent 1st order responses at the top (Fig. 19A) and 2nd order responses on the bottom (Fig. 19B) for the experimental eye undergoing progressive sensitivity losses (the MD at each recording session is indicated at the top of each column). The mfERGs from the control eye, which did not change from session to session, were quite similar to the data obtained in the initial session and showed marked oscillatory waves in the records. The control and early glaucoma responses (left column) also showed distinctive nasotemporal variations; for the 1st order responses, the recordings from the upper and lower nasal fields (UN and LN) exhibit a clear drop in potential after the large positive peak, whereas the temporal (UT and LT) visual field recordings decayed more slowly from the peak. In the 2nd order responses, the prominent negative deflections were larger in the nasal retina than in the temporal retina. As illustrated by the data obtained at later times with more pronounced visual field defects, as the losses in visual sensitivity became greater, the 1st order responses became progressively smoother, the nasotemporal differences disappeared from the 1st and 2nd order responses, and 2nd order responses became very small.

Investigations of the variation of the mfERG waveform as a function of retinal eccentricity were conducted by pooling responses of stimuli from a series of five concentric rings that are illustrated by the stimulus pattern in Fig. 20 (Frisman et al., 2000). The mfERG data that are presented in Figs. 20A and B illustrate the control data and the effects of progressive changes in the 1st (A) and 2nd order (B) recordings from a glaucomatous eye. The four records in each column were derived from the central stimulus and the three innermost rings; results from rings 4 and 5 are not presented because the waveforms closely resemble those from ring 3. The comparison of mfERGs across retinal eccentricities indicates that the effects of experimental glaucoma were diffuse, affecting central and more peripheral ERG responses similarly. However, as visual sensitivity deteriorated with experimental glaucoma, there was a progressive smoothing of the 1st order responses and reduction in amplitude of the 2nd order responses. The data in this figure also reveal that glaucoma has a large effect on responses near the fovea. In addition, when the visual sensitivity was the most reduced (rightmost column) the waveforms of the mfERGs at all eccentricities became very similar.

The most obvious changes in the mfERG associated with the advancing visual field defects are the loss of oscillatory potentials. A power spectrum analysis of the 1st order responses of the mfERG of the control data compared to the final data (last column) indicated that the response to the central hexagon contained the most power at high frequencies in the control records, with a substantial reduction in the power at frequencies greater than 60 Hz for the glaucomatous eye (Frisman et al., 2000). However, further quantification of the mfERG responses at high frequencies during glaucomatous neuropathy is required to understand the significance of these changes.

An issue for objective monitoring of the progression of glaucomatous optic neuropathy by mfERG is whether changes in the mfERG waveform correlate with perimetric measurements. Correlated measurements by objective and subjective methods would have important implications for clinical and experimental work, but the issues are complex. Although some components of the mfERG waveform are systematically reduced with loss of ganglion cells from experimental glaucoma (Hare et al., 2001), studies of clinical glaucoma have not shown mfERG changes that are correlated to the localized defects found by perimetry (Klistorner et al., 2000; Fortune et al., 2001). However, these cross-section studies of stages of glaucoma may have greater variability than for measurements of the changes over time during the progression of neuropathy for an individual subject.

Preliminary investigations of the progression of experimental glaucoma on the mfERG were conducted
in conjunction with the study of the PhNR, using the same animals and the same recording sessions for both ERG measurements. The changes in mfERG response density were based on the P1N2 amplitude of the waveform, i.e., the amplitude between the first positive (P1) peak at around 35 ms from the beginning of the record, and the second negative (N2) peak at about 40 ms. The P1N2 amplitudes were derived from the data averaged across the three central rings of mfERG stimuli (see Fig. 20) which was necessary to clearly identify the main positive peak amid the oscillations. For each session, the P1 N2 amplitudes were compared to the MD perimetry index as an indicator of the generalized loss of sensitivity across the visual field.

The data for the P1N2 amplitudes are presented in Fig. 17C in the same format as the data for the PhNR in Fig. 17B. In contrast to the PhNR, experimental glaucoma did not cause a reduction in the amplitude of the P1N2 until the visual field defects were highly significant. With more advanced visual defects, the ERG reductions were linearly related to the MD of the visual fields. On the basis of these preliminary data, it appears that the mfERG may be more useful as an objective measure of advanced glaucomatous neuropathy than for its early detection. In this respect, the data from the two ERG procedures may be in agreement with the general concept that the best techniques for early diagnosis may not also be the best techniques for determining progression, especially in advanced cases. The differences in the PhNR and mfERG are apparent in a comparison of the data in Figs. 17B and C. The most significant reductions in PhNRs are associated with mild...
visual field defects (MD < 6 dB), while the more significant reductions in P1N2 occur in the presence of advanced defects (MD > 6 dB).

Although there are several other properties of the waveforms in the mfERG that can be analyzed, data for the P1N2 amplitude were presented because it was better correlated with the visual fields data than other measures. Specifically, the two measures of the mfERG that were best correlated with ganglion cell loss in experimental glaucoma, i.e., the N2P2 amplitude of the 1st order response and the P1N1 amplitude of the 2nd order response (Hare et al., 2001), were not as well correlated with field loss. However, confirmation of these trends will require studies of more animals and additional studies of the MD as an index of the retinal ganglion cell losses from glaucoma.

Altogether, the investigations of ERGs in experimental glaucoma have produced clear support for the application of the ERG for an objective measure of the stage of pathophysiology of glaucoma. Components of the ERG waveforms that were generated by the retinal ganglion cells were identified with each method, but some methods, such as the STR and photopic pattern-ERG, are technically more demanding for general clinical procedures, although they are viable research methods. On the other hand, the photopic full-
field flash ERG and mfERG procedures both are likely candidates for use in patient care or for clinical investigations and each may be more useful at different stages of glaucoma. The differences on the form of correlation between ERG amplitudes and perimetry MD indices for the two procedures suggest that the structure–function relationships for the responses may be different, with the PhNR amplitude affected more by early losses of ganglion cells and the mfERG affected more by advanced losses of ganglion cells. For example, if it is assumed that the amplitude of the PhNR represents the linear sum of signals from all of the ganglion cells, then it is expected that it would be significantly reduced by a 40–50% loss of ganglion cells, whereas visual sensitivity is not. On the other hand, the mfERG response to sequential changes in contrast would be influenced by response probability considerations, in the same way as visual sensitivity.

Neither type of ERG produces discrete responses that can be associated with the localized visual field defects that are typical of perimetric measures of visual sensitivity in glaucoma. In this sense, the ERG amplitude has a similarity to the global indices from perimetry data that are calculated to derive a single value for the stage of functional deficit. Specifically, the ERG data can be considered as global measures of retinal function in the same way that the MD value is considered an indication of the diffuse loss of visual sensitivity. However, it is important that the continuing studies on experimental glaucoma demonstrate that these ERG procedures provide accurate and precise reflections of the overall state of ganglion cell loss, so that the information about the regional losses is not essential.

19. Summary and conclusions

The functional effects of ganglion cell death in glaucoma have been studied by diverse methods with converging results. Each of the studies used a common method of behavioral perimetry to assess the clinical stage of visual field loss from experimental glaucoma to relate the separate investigations of the structure–function relationship. The principal studies addressed: (1) the psycho-physiological links between ganglion cell loss and the depth of visual field defects, (2) the relative metabolic effects of glaucoma on neurons in the parallel afferent M- and P-pathways to the visual cortex, and (3) scotopic and photopic ERG measures of optic neuropathy from glaucoma. The overall findings have shown that the loss of visual sensitivity from glaucoma is best explained by the reduction of probability summation among stimulus detector mechanisms. The general progression in the loss of sensitivity from the mid-peripheral to more central retina produces concurrent decreases in metabolic products along the afferent visual pathway that are not selective to either of the M- or P-cell parallel processing streams. Thus, these results suggest that the accuracy and precision of clinical perimetry should be improved by employing stimuli that are specific to smaller numbers of detecting mechanisms in either the M- or P-cell pathway. Alternatively, in lieu of subjective testing, objective methods for diagnosing and assessing progression of glaucoma, such as ERG recordings, may be employed. Several ERG methods are capable of isolating a ganglion cell component, but the two that appear to have the best potential for clinical and research utility are the PhNR and the mfERG. Interestingly, the preliminary comparisons of these techniques also suggest that each might have better sensitivity at a different stage of optic neuropathy. In fact, this result may be in agreement with a more general concept that different subjective or objective procedures should be optimal for the clinical management of early vs. advanced glaucoma.

As a whole, these studies demonstrate that experimental glaucoma in monkeys is a unique model for investigations of the psychophysical, electrophysiological, histochemical, and/or anatomical characteristics of a prevalent ocular disease. In the monkey-model, laser-induced ocular hypertension is the sole factor in the development of the glaucomatous optic neuropathy and, because the intraocular pressure is generally quite high, the full extent of the damage occurs in a period of a few months. The experimental condition eliminates all but one of the well-known epidemiological risk factors for clinical glaucoma, while all other aspects of cell death and clinical presentation appear to be identical in experimental glaucoma and clinical glaucoma. Therefore, although experimental glaucoma cannot be used for studies of the mechanisms by which factors such as age, race, genetics, etc., increase the probability of acquiring glaucoma, it is a valid model for any of the causes/effects associated with neural losses from glaucoma. Furthermore, because the visual systems of macaque monkeys and humans are practically identical, the results from the array of experiments that have been described can be applied directly to glaucoma in patients.

Acknowledgements

This study was supported in part by a research grant from Alcon Research, Ltd., Fort Worth, TX, and National Institutes of Health/National Eye Institute grants RO1 EY01139, RO1 EY 03611, EY 06671 and P30 EY0551 to the University of Houston and grants RO1 EY07751, RO1 EY11545, and P30 EY10608 to the University of Texas–Houston. Additional support was
References


