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ORIGINAL RESEARCH ARTICLE

Novel electrophysiological instrument for rapid and objective assessment of magnocellular deficits associated with glaucoma

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Abstract *Purpose* To introduce a rapid and objective electrophysiological technique that can assess visual function in the magnocellular pathway, which is thought to be affected in early-stage glaucoma. *Methods* Low-contrast bright or dark isolated-checks were luminance-modulated against a static background at 10 Hz in order to drive preferentially the magnocellular ON or OFF pathway. Visual evoked potentials were recorded during 1-s epochs of stimulation and responses at the stimulus frequency were measured. Artifact rejection features ensured that eight valid runs were obtained per eye. Signal-to-noise ratios (SNR) were derived based on a multivariate statistic. In order to demonstrate its functionality, a small group of patients with glaucoma ($N = 18$, Snellen acuity of 20/30 or better) and control observers ($N = 16$) were tested. A participant failed the test if either eye yielded an SNR ≤ 1. Receiver-operating-characteristic curve analysis was used to estimate the accuracy of group classification. *Results* The instrument was found to elicit reliable responses from control observers. For the 15% bright condition, all control observers yielded significant isolated-check VEPs (icVEPs), whereas the majority of patients failed to do so, indicating significant losses in central visual function. This condition produced the highest classification accuracy (94%), followed by the 10% dark condition (91%). *Conclusions* Both ON and OFF divisions of the magnocellular pathway can be assessed rapidly through the application of the icVEP technique. This measure of central visual function may be of value in the detection of glaucomatous deficits and may complement tests of peripheral function.

Keywords Glaucoma · Magnocellular pathway · ON and OFF pathways · Visual evoked potentials

Abbreviations

CRT	Cathode ray tube
DAC	Digital-to-analog converter

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DFT	Discrete Fourier transform
FDT	Frequency doubling technology
FFC	Fundamental frequency component
HVF	Humphrey visual field
IcVEP	Isolated-check visual evoked potential
IOP	Intraocular pressure
M	Magnocellular
MD	Mean deviation
MfVEP	Multifocal visual evoked potential
P	Parvocellular
PERG	Pattern electroretinogram
POAG	Primary open-angle glaucoma
ROC	Receiver-operating-characteristic
SAP	Standard achromatic perimetry
SNR	Signal-to-noise ratio

Introduction

Glaucoma is one of the leading causes of blindness in the United States, and it is characterized by gradual and progressive degeneration of retinal ganglion cells. Unfortunately, by the time this glaucomatous neuropathy is detected, there is typically permanent damage to the visual system and extensive loss of visual function [1]. The detection of early glaucomatous damage is therefore of critical importance. A number of ganglion cell subpopulations exist in the retina, and it is possible that activity in one subpopulation may be more sensitive to early-stage glaucoma than activity in the other subpopulations. These subpopulations represent parallel pathways for the transmission of visual information to the brain, and there is evidence that glaucomatous neuropathy extends to primary visual structures in the brain [2].

The dichotomy of ON and OFF pathways, which appears to govern the separate perceptions of brightness and darkness, has long been established. [3–5] More recently, the functional distinctions between magnocellular (M) and parvocellular (P) pathways (each one with ON and OFF subdivisions) have become clear [6]. The M pathway conveys primarily low spatial frequency/high temporal frequency information, and it is sensitive to low levels of luminance contrast but rather insensitive to chromatic information. On the other hand, the P pathway is capable of transmitting higher spatial frequency information, but at somewhat lower temporal frequencies, and it is not sensitive to low levels of luminance contrast but quite responsive to isoluminant chromatic signals [7, 8]. The neurons contained within each of these parallel pathways differ in structure as well as function. It has been suggested that disease processes in glaucoma may have differential effects on these independent streams. Assessment tools that are capable of tapping select pathways, therefore, should improve our ability to detect early dysfunction in the system.

One hypothesis, based on histological evidence, is that ganglion cells with large diameter axons are preferentially damaged in early glaucoma (presumably those in the magnocellular pathway) [9, 10]. An experimental primate model of glaucoma, however, has produced conflicting results [11]. Based on this hypothesis, though, new techniques have been introduced to the field that are designed to measure M pathway function. Currently, the most promising of these diagnostic devices available in the market is frequency doubling technology (FDT). Although the name implies that the commercial instrument assesses the integrity of a perceptual phenomenon discovered by Kelly [12], in fact, it measures contrast sensitivity in the low spatial frequency and high temporal frequency region of the visual system. Even though the actual frequency doubling percept appears to result from cortical function, the measure used in the commercial instrument probably reflects the activity in the M pathway [13]. The advantage of the FDT technique over other existing ones is the relatively brief duration of the test (approximately 5 min per eye for the full threshold test) [14]. One disadvantage of FDT is the difficulty of the behavioral task, and there are discrepant reports of its sensitivity for the early detection of glaucoma [15]. Also, its reliability and validity remain to be established. Of course, the current gold standard, visual field testing based on standard achromatic perimetry (SAP) is also variable with only moderate test-retest reliability [16] and it is a time-consuming and often difficult task for the patient.

Additional functional measures applied to the diagnosis of glaucoma are the multifocal visual evoked potential (mfVEP), which is an electrophysiological visual field test, and the pattern electroretinogram (PERG). The mfVEP, although more time consuming to administer than a conventional visual field test [16–19], is a promising technique for detection of glaucomatous damage. The PERG technique has the advantage of a shorter testing time (approximately

3 min of recording time), but it does not provide topographic information [20, 21].

The instrument introduced in this article utilizes a novel version of the isolated-check VEP (icVEP) technique [22–27] and is designed to tap cortical activity initiated primarily by afferents in the M pathway (both ON and OFF subdivisions). In a recent pilot investigation, we were able to demonstrate complete separation in responses of primary open-angle glaucoma (POAG) patients ($n = 10$) and age-similar controls ($n = 10$) [28]. Although the sample size is small, the accuracy of 100% is encouraging and prompted the development of a more efficient and simpler device to probe glaucomatous deficits in the present study. The conditions used in the Badr et al. [28] study were slight modifications of conditions used by Greenstein et al. [26], which yielded results that did not achieve complete separation of the glaucoma and control groups, but did reach 93.3% accuracy for the critical condition. Thus, the objective and efficient icVEP instrument described below is expected to aid in the early detection of glaucoma.

Materials and methods

Participants

A total of 34 participants met the inclusion/exclusion criteria and were selected to evaluate the operation of the instrument. Of these participants, there were 18 patients with a diagnosis of glaucoma (17 open-angle, 1 angle-closure) and 16 controls. All participants had best corrected visual acuity of 20/30 or better. The control group had a mean age of 52.4 (SD = 10.0) and the patient group had a mean age of 66.2 (SD = 7.5). Given that there is a significant difference in age between the groups, additional analyses were performed with an older subset of the control group ($M = 60.9$, SD = 8.5, $N = 7$) in which no significant difference in age existed. Also, correlational analyses were performed to explore the relations between age and the response measures for each complete group. Glaucomatous optic neuropathy was defined as either cup/disc asymmetry between fellow eyes of greater than 0.2, rim thinning, notching, excavation, and/or retinal nerve fiber layer defects as defined by a loss in mean retinal nerve fiber layer thickness measured with optical coherence tomography (Stratus OCT 3, Carl Zeiss Ophthalmic Systems, Inc., Humphrey Division). Each patient had at least one eye with glaucomatous damage, as defined by an abnormal optic disc seen on slit lamp examination and stereoscopic disc photography, with a corresponding repeatable visual field defect on SAP and glaucoma hemifield test outside normal limits. The glaucoma cases had Humphrey visual field (HVF) 24-2 tests performed. These patients had a wide range of MD values obtained from SAP. Based on the more affected eye, there were 11 patients with less than 6 dB loss compared to controls, three patients with a loss of 6–12 dB, and four with a loss greater than 12 dB. No specific intraocular pressure (IOP) was required in order for a diagnosis of glaucoma to be made. Patients were excluded from the study if they had, diabetes, significant cataract, unequal pupil diameters and pupil diameters <2.0 mm (as measured by the HVF analyzer), refractive errors exceeding ± 5.00 diopters sphere or 2.00 diopters cylinder, and ophthalmic diseases other than glaucoma. The control observers had no history of ocular disease, IOP $\leq$ 21 mm Hg, normal optic disc appearance based upon clinical examination by a glaucoma specialist using a 78 D lens, and normal SAP. Absence of glaucomatous optic neuropathy was defined as vertical cup-disc asymmetry <0.2, cup-disc ratio ≤ 0.6, and intact neuroretinal rim without peripapillary hemorrhages, notches, localized pallor, or nerve fiber layer defect.

Stimuli

A pilot experiment was performed to select the stimulus parameters to include in the prototype instrument used in the present study. Differences in the hardware of this instrument as compared to the research equipment used in the previous investigations [26, 28] necessitated a reexamination of the appropriate stimulus conditions for favoring M pathway stimulation. The present device used a standard video card with 8-bit digital-to-analog converters (DACs) per electron gun (R, G, and B) and a 60 Hz frame rate, as compared to 12-bit DACs and 119 Hz frame rate in the former research equipment. Examination of the pilot data led to the selection of a sinusoidal temporal signal (6 frames/cycle) of 10 Hz and two nominal contrast levels (10 and 15%) in order to elicit significant VEPs consistently in control observers. Both positive- (bright) and negative-contrast (dark)

conditions were included in the test protocol. In order to assess the performance of the video board used (256 gray levels), luminance at each gray level was measured with a PhotoResearch Model 1980B scanning spectroradiometer to determine the actual Weber contrast of the stimulus. For the maximum positive contrast used in the study (15.36%), the linear relation between the luminance of the CRT display and gray level explained 99.79% of the variance. For the maximum negative contrast used in the study (−15.10%), the linear relation between the luminance of the CRT display and gray level explained 99.86% of the variance. The 7–8 s swept-parameter runs in which contrast was increased in 1-s octave steps, used in previous work [26–28], were replaced with 2-s runs: in the 1st second, half the test contrast level was presented as an adaptation condition, and in the 2nd second, the full test contrast was presented to elicit the desired VEP. An auditory signal preceded the start of a run, at which point a uniform field with a fixation cross was replaced by a patterned field with a fixation cross half the initial size. This auditory-visual cue facilitated careful fixation on the center of the screen. The spatial pattern used, based on the pilot results, was a 24 × 24 array of isolated-checks (individual squares embedded in a uniform background field, 14 × 14 min of arc per square). It subtended 11° at the viewing distance of 114 cm. An example of the dark-check pattern as presented on the display monitor is depicted in Fig. 1. The luminance of the display's static background was 51 cd/m^2. Each pattern was presented in appearance–disappearance mode with Weber contrast modulated by the temporal sinusoid (depicted in Fig. 2). Weber contrast (C_W) was defined by the following equation:

$$C_W = \frac{L_c - L_b}{L_b}$$

where L_c is the luminance of the checks at their maximum difference from background luminance, and L_b is the luminance of the static background.

Electrophysiological recording and analysis

Gold-cup electrodes filled with electrolytic paste were placed at the following midline sites on the scalp, based on the international 10–20 system [29], to comprise a single electrophysiological channel: O_z (occipital) referenced to C_z (vertex) with floating ground at P_z (parietal). The differential amplifier used had a gain of 10,000 and a filter with a bandwidth from 0.5 to 100 Hz. The electroencephalographic (EEG) signals were recorded at a sampling rate of 120 Hz, and the sampling was synchronized with the stimulus frame rate (60 Hz) in order to eliminate side-lobe leakage contamination of the fundamental response component from other harmonic frequency components. A discrete Fourier transform (DFT) was performed on the EEG data to calculate the fundamental frequency (10 Hz) component of the icVEP,

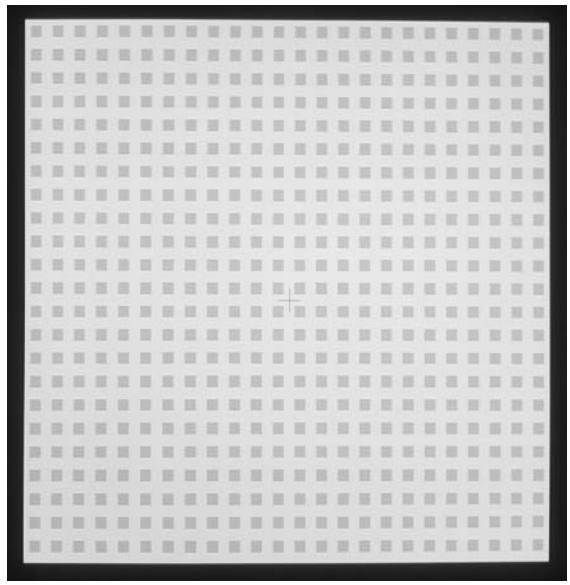

Fig. 1 Example of a dark-check condition (negative contrast)

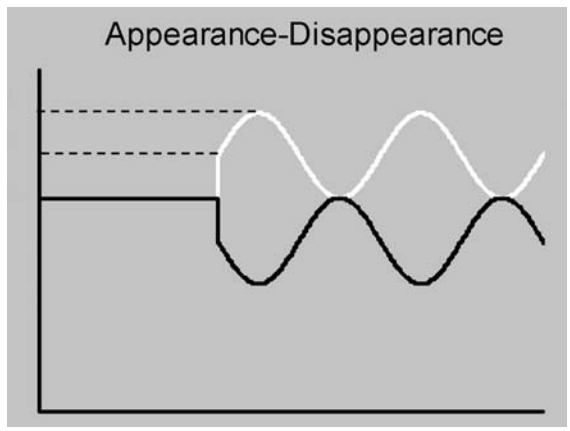

Fig. 2 Luminance of checks was modulated sinusoidally at 10 Hz either above the static background field (bright condition) or below it (dark condition) such that the pattern disappeared at one point in time during each cycle of modulation

which was the dominant frequency component in the response given the stimulus conditions used.

The VEP measured from the scalp is usually a signal smaller than 10 μV peak-to-peak with relatively large variability, associated with eye movements and other (neural and non-neural) sources. In order to obtain reliable VEPs, three techniques were employed during the test: automated noise detection, automated outlier analysis, and operator verification.

The noise detection and outlier analysis features are incorporated in the program and they are capable of detecting 60 Hz line noise and saturation in the EEG recording. If noise is detected, the EEG epoch is rejected, and the program will prompt the operator to repeat the run. If the run is determined to be valid, the EEG data will be displayed on the operator's monitor, and the operator is prompted to either accept or reject the data based on inspection for additional artifacts and based on whether proper fixation was maintained during the run. If the data are accepted, the program will instruct the operator to initiate the next run until a set of eight valid runs are accumulated.

The built-in outlier analysis algorithm determines if one of the runs produced a fundamental frequency component (FFC) that is an outlier relative to the remaining seven FFCs based on a statistical criterion. If an outlier is identified, the program will discard that FFC and the program will prompt the operator to repeat the run until eight qualified runs are collected. Given these eight FFCs, the program calculates the mean FFC and the radius of a 95% confidence circle using the T^2_{circ} statistic [30]. The signal-to-noise ratio (SNR) was defined as the ratio of the mean amplitude of the FFC to the radius of the 95% confidence circle, and it is used to assess the reliability of the VEP. Thus, SNR > 1 indicates a significant response at the 0.05 level. At the end of the test, the individual and mean FFC values, confidence circle, and SNR are displayed on the operator's monitor. (Typically, a test can be completed in less than 1 min). The SNR of the FFC is used to predict the presence of glaucomatous damage. Receiver-operating-characteristic (ROC) curve analysis was used to estimate the accuracy of prediction, based on a nonparametric measure, A′ [31], given each stimulus condition under study. The 95% confidence limits for the area under the ROC curve were calculated using the SPSS 12.0 statistical package based on the 2×2 contingency table.

Procedure

This research followed the tenets of the Declaration of Helsinki. All participants signed the consent form approved by the Institutional Review Board at New York Presbyterian Hospital—Columbia University Medical Center after reading it and listening to an explanation of the study and its possible consequences. Each participant received a full ophthalmic examination from one of the collaborating glaucoma specialists. This included refraction, visual acuity, intraocular pressure, slit lamp biomicroscopy, stereoscopic optic nerve head photography, and SAP using a Humphrey Visual Field Analyzer II (24-2 SITA standard program).

For the VEP procedure, surface electrodes were applied to the scalp with water-soluble paste. The observer was instructed to listen for the auditory signal and then fixate the cross in the center of the display carefully for the 2-s period during which the pattern was displayed and the EEG was recorded. Typically, application/removal of electrodes and instructions consumed less than 5 min. Each subject wore the appropriate optical correction for the viewing distance of 114 cm.

A flow chart was used to facilitate the operator in following the proper test protocol. If a participant failed on one stimulus condition (SNR ≤ 1), the operator initiated a retest on that condition. If the participant failed a condition twice, the operator advanced to the next condition in the flow chart. The order of stimulus conditions tested was as follows: 10% positive contrast, 15% positive contrast, 10% negative contrast, 15% negative contrast.

Results

Descriptive statistics

Age

The median and range of age in years for each group of observers analyzed was as follows: controls: 52 (40–73), glaucoma patients: 67 (54–77).

Intraocular pressure

Control observers ranged from 13 to 19 mm Hg and glaucoma patients ranged from 7 to 23 mm Hg.

Lower pressures in the latter group are likely associated with pharmacological treatment.

Pupil area

Retinal illuminance depends on area of the pupil and it is known to influence responses in the magnocellular pathway [32]. Swanson et al. [33] have shown that FDT perimetry depends critically on retinal illuminance. In our sample of participants, pupil area ranged from 7.6 to 32.2 mm^2 in controls (pupil diameter OD: $M = 5.06$ mm, SD = 0.80; OS: $M = 4.90$ mm, SD = 0.83) and 7.1 to 32.2 mm^2 in glaucoma patients (pupil diameter OD: $M = 4.93$ mm, SD = 0.97; OS: $M = 4.59$ mm, SD = 0.87). No significant difference between groups was found in either monocular analysis: OD $t(21) = 0.337, P = 0.739$; OS $t(21) = 0.816$, $P = 0.424$. Correlational analysis of pupil area with the icVEP response measures did not reveal a critical dependence over the range explored ($P > 0.05$, for all conditions and both eyes).

Humphrey visual field: mean deviation (MD)

Correlational analysis of MD values with the icVEP response measures did not reveal a critical dependence for any of the conditions and eyes tested ($P > 0.05$).

Isolated-check visual evoked potential (icVEP) data: individual plots

Each dataset of eight fundamental frequency responses to each stimulus condition is displayed in a sine versus cosine coefficient plot for each participant. Figure 3 shows representative results (15% bright check condition) for one control and one glaucoma patient. The circle that surrounds the mean of the eight individual responses represents the 95% confidence region based on the T^2_{circ} statistic [30]. Signal-to-noise ratio (SNR) is 1.85 for the control observer and 0.50 for the patient. When the circle is far from the origin, the SNR is well above 1, which is the critical value for significance at the 0.05 level. When the circle encompasses the origin, the SNR is below 1 and the signal is smaller than the noise level (i.e., no significant response). It should be noted that the visual acuity for the glaucoma patient as well as for the control was 20/20.

15% Positive-contrast (bright) condition

Based on previous research from our group [26, 28], we expected responses associated with stimulation of the magnocellular (M) ON pathway to be most affected in the early stage of glaucomatous neuropathy. Thus, the bright-check conditions were considered the most promising in the present study. Although the previous work demonstrated excellent separation between patient and control groups when the stimulus condition was 8% positive contrast and 12 Hz temporal modulation, the work was performed with research equipment that had enhanced temporal and gray-level resolution as compared to the current prototype. Pilot testing during the present study indicated that slightly different stimulus parameter values would be needed to yield significant responses consistently from control observers; that is why we selected 10 Hz sinusoidal

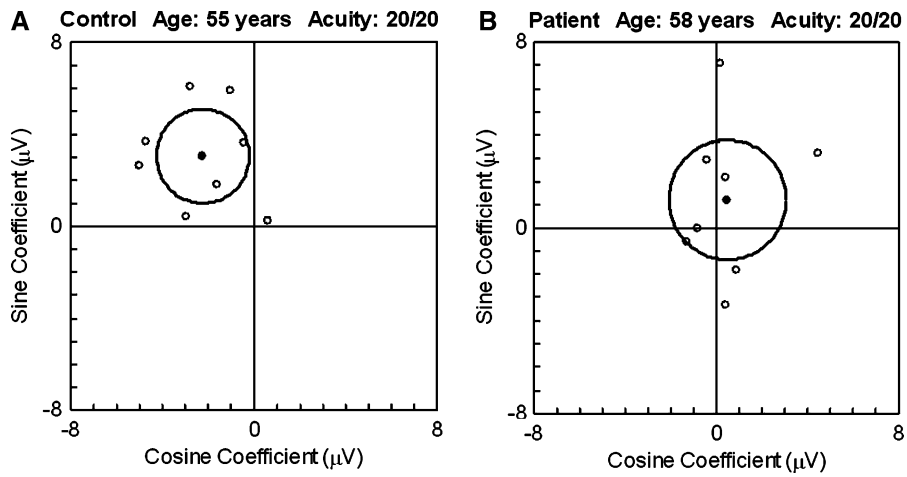

Fig. 3 Plot of sine versus cosine coefficient for the fundamental frequency component of the icVEP under the 15% bright-check condition for (**a**) one representative control (left half; SNR = 1.85) and (**b**) one glaucoma patient (right half; SNR = 0.50)

temporal modulation and contrast levels of 10 and 15%. Results with the 10% positive-contrast condition demonstrate that several controls generated SNRs for at least one eye that were below the criterion value of 1. We focused our analysis, therefore, on the 15% positive-contrast condition. A participant was deemed to fail the test (positive test result) if either eye yielded an SNR value of 1 or less. SNR for the left eye (OS) is plotted versus SNR for the right eye (OD) for the 15% positive-contrast condition for both control and glaucoma groups in Fig. 4. Here, the criterion SNR is indicated by a vertical line for OD and a horizontal line for OS. Note that all control data (open circles) fall within the pass region, the upper right quadrant of the plot, and the majority (all but four) of the patient data points (open triangles) fall within the fail regions. Classification of participants according to test results is indicated in the 2 × 2 contingency table (Table 1). Given the SNR criterion of 1, the sensitivity of the test is 78% and the specificity is 100%. An ROC analysis (Fig. 5a) indicates that the a priori SNR criterion of 1 is optimal for discrimination of patients and controls. A nonparametric estimate of the area under the ROC curve, A′ [31], yields a measure of accuracy of the screening test of 94%. This is encouraging given that the majority of glaucoma patients tested (11 of 18) had mean deviation scores of less than 6 dB loss on the 24-2 SITA-standard Humphrey visual field test. The 95% confidence interval for the area under the ROC curve is 0.74–1.00.

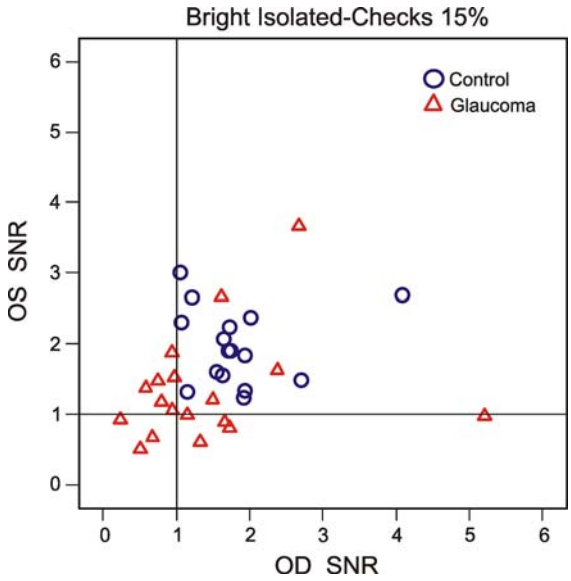

Fig. 4 Plot of signal-to-noise ratio (SNR) for left eye (OS) versus right eye (OD) data collected from controls and glaucoma patients under the 15% bright condition

Table 1 Contingency table of group membership versus test result for the bright 15% condition

Stimulus: B 15%	Glaucoma (D+)	Control (D−)
icVEP Test +	14	0
icVEP Test −	4	16

10% Negative-contrast (dark) condition

Typically, dark checks elicit larger VEPs than do bright checks (which appears to reflect greater contrast gain for the OFF relative to the ON pathway [22]. In this study, the 10% dark stimulus condition produced significant responses in 14 of 16 control observers (SNR > 1), but in only 3 of 18 glaucoma patients (Fig. 6). These data yielded a sensitivity of 83%, a specificity of 88%, and an estimate of area under the ROC curve (A′) of 91% (Fig. 5b), which suggests that deficits associated with stimulation favoring the magnocellular OFF pathway may also be indicative of early glaucomatous damage. The 95% confidence interval for the area under the ROC curve is 0.74–1.00.

10% Positive-contrast (bright) and 15% negative-contrast (dark) conditions

The remaining two stimulus conditions yielded less accurate results than did the 15% bright and 10% dark conditions. The 10% bright condition yielded an accuracy (A′ estimate of area under the ROC curve) of only 73% due to poor specificity (56%), with sensitivity of 72% (ROC curve illustrated in Fig. 7a). The 15% dark condition yielded an accuracy of 82% due to poor sensitivity (56%), with specificity of 88% (ROC curve illustrated in Fig. 7b).

Correlations of signal-to-noise ratios between the critical test conditions

Pearson correlation coefficients were calculated for SNR values obtained under the critical (bright 15% versus dark 10%) stimulus conditions for each eye and for each group tested. The control group yielded no significant correlations between the responses to

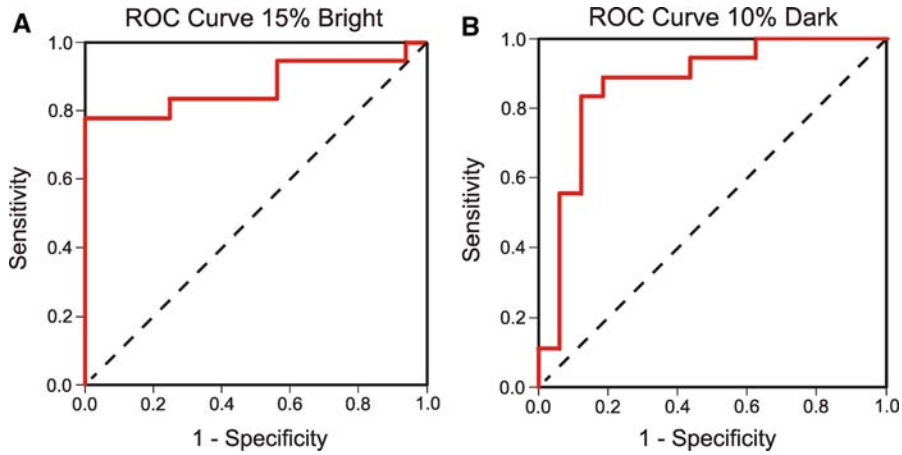

Fig. 5 Receiver-operating-characteristic (ROC) curve for data collected under the (**a**) 15% bright condition and (**b**) 10% dark condition

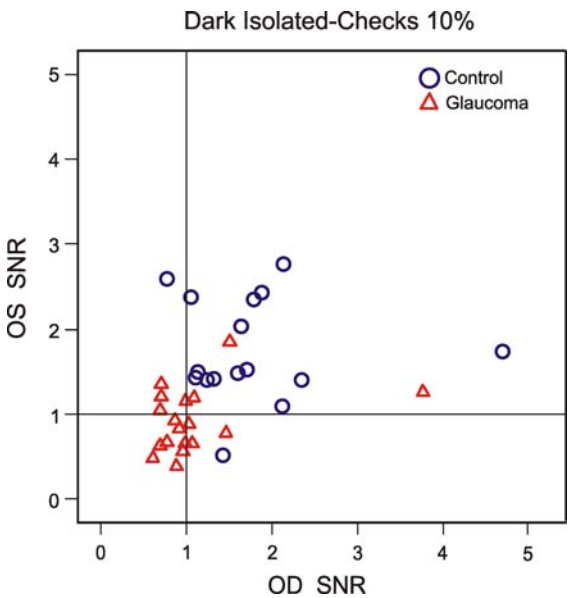

Fig. 6 Plot of signal-to-noise ratio (SNR) for left eye (OS) versus right eye (OD) data collected from controls and glaucoma patients under the 10% dark condition

bright 15% versus dark 10% conditions for either eye tested. The glaucoma group, however, did yield significant correlation coefficients between responses to these two conditions for both right ($r = .78$, $P < .0005$) and left ($r = .48$, $P < .05$) eyes.

Associations of age with signal-to-noise ratios and classification accuracy

Correlational analyses were performed between age and SNRs for each group under the two critical test conditions. For the entire control group, there were no significant correlations of SNR with age for either eye tested under either of the critical test conditions, even though age ranged from 40 to 73 years. Similarly, the patient group did not yield any significant correlations between SNR and age.

Classification analyses were repeated with a subset of older controls which did not differ significantly from the patient group in age ($t(23) = -1.5$, $P = 0.18$). In this analysis, the bright 15% condition yielded a sensitivity of 78%, specificity of 100%, and accuracy of 94%, which is unchanged from the analysis based on the entire control group. The dark 10% condition yielded a sensitivity of 83%, specificity of 86%, and accuracy of 91%, which is the same as the analysis based on the entire control group with the exception of a 2% difference in specificity.

Discussion

The 15% positive-contrast (bright) condition and 10% negative-contrast (dark) condition produced promising results. Even though the majority of patients had mean deviation scores of less than 6 dB with little or no functional deficits in central vision as determined by visual acuity and visual field tests, the rapid and objective icVEP test introduced here suggested significant magnocellular deficits. The results indicate that both ON and OFF divisions of the M pathway appear to be affected in early glaucoma. The greater accuracy associated with the icVEP test under the bright condition supports the findings of previous work [26, 28], which suggested that the M-ON pathway was affected in an early-stage of

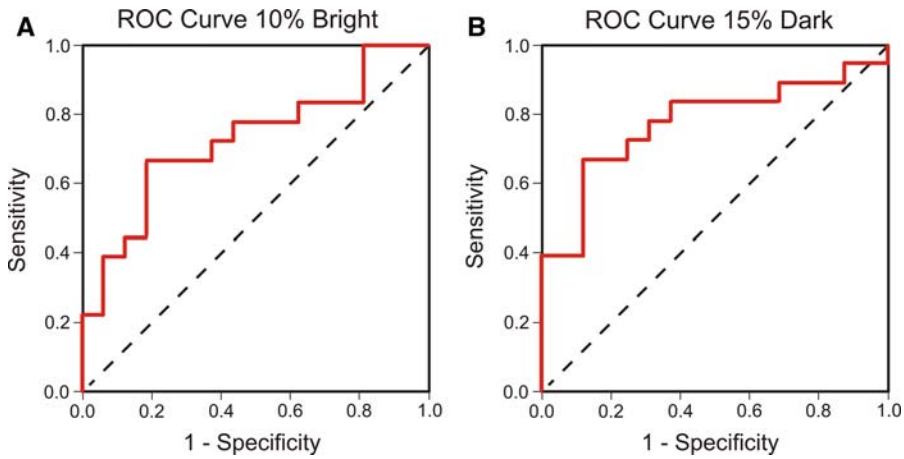

Fig. 7 Receiver-operating-characteristic (ROC) curve for data collected under the (**a**) 10% bright condition and (**b**) 15% dark condition

glaucoma. It is of interest to note that, in humans, M cells are considerably larger than P cells, and ON cells are larger than OFF cells [34]. Thus, these results are consistent with histological work that supports previous findings of preferential death of retinal ganglion cells with large diameter axons in glaucoma [10]. It should be noted, however, that koniocellular neurons might also be affected in early-stage glaucoma as evidenced by deficits detected with short-wavelength automated perimetry [35] and neuropathological findings using an experimental primate model [11]. Small P cells appear to be involved in glaucoma as well, but a VEP investigation that compared responses elicited by isoluminant red checks to luminance-contrast conditions similar to those used in the present study also demonstrated higher diagnostic yield with low contrast bright checks [26]. It is important to point out that given the small sample size in the current study and the similar accuracy of classification obtained with the bright 15% and dark 10% conditions, this preliminary study does not provide strong evidence for a differential effect of glaucoma on ON versus OFF cells.

Findings from studies that used an experimental primate model do not demonstrate a selective loss in magnocellular as compared to parvocellular neurons [11]. It should be noted, however, that several differences exist between true glaucoma and the experimental monkey model. In addition to likely differences in the mechanisms affected by the actual condition as compared to the artificially induced condition, there is a critical difference in human versus monkey M cells. It has been shown that M cells in humans are considerably larger than those in monkeys, whereas P cells have been found to be of about equal size in the two species [34]. Thus, the morphologic data, as mentioned above, indicate that the M-ON cells in humans are larger than any cell type found in monkeys. It is possible, therefore, that a disease process that affects large cells preferentially would produce more pronounced selective deficits in humans. In addition, a functional measure of select cortical activity (e.g., the icVEP) might be a particularly sensitive indicator of a functional deficit in the large (M) cell pathway prior to any observable structural damage. In fact, a recent report on the first clinicopathological case of human glaucoma that showed neural degeneration in the lateral geniculate nucleus and visual cortex noted that the size (cross-sectional area) of magnocellular neurons in the case of glaucoma was significantly smaller ($P < 0.0001$) than that in age-matched controls [2].

Typically, the ON pathway has lower contrast gain than the OFF pathway [22], and therefore, higher contrast (15%) was required to elicit significant responses from control observers and yield high test specificity (100%). The 94% accuracy (estimated area under the ROC curve) associated with this test condition is superior performance as compared to other existing functional measures of glaucomatous damage (especially given that most of the glaucoma patients had relatively small mean deviation scores with standard achromatic perimetry). (Unfortunately, the measure against which the icVEP results are evaluated is less than an ideal standard.) Although the M-OFF test (10% dark condition) yielded a slightly lower estimate of accuracy (91%), it still performed well. Thus, the rapid and objective icVEP test

appears to meet the requirements for an assessment instrument for the early detection of glaucoma.

It should be pointed out that alternative explanations for the functional glaucomatous deficits observed in the present work are possible. For example, loss of afferent input to primary visual cortex due to glaucomatous neuropathy would result in decreased conductance of cortical neurons. These cells, therefore, would exhibit longer integration time constants and thus decreased responsivity at high temporal frequencies [27]. Regardless of which explanation ultimately is found to correctly account for the present results, however, the fact remains that the icVEP test under the critical stimulus condition provides a sensitive measure in these cases of glaucoma.

The sampling process resulted in a control group that was significantly younger than the patient group, even though the same inclusion/exclusion criteria for age were applied. Additional analyses were performed for a possible confound due to this age difference. Results demonstrated that within the age range of interest (40–77 years) there was no association of the response measure (SNR) and age and no explanatory power of age on the classification accuracy for the critical test conditions.

Earlier studies used a swept-parameter paradigm that increased the contrast of the isolated-check pattern in 1-s octave steps [24, 25]. The duration of a single run was 7–8 s in duration. The prototype instrument developed in the present study uses runs of only 2 s in duration, which reduces test time and greatly increases the feasibility of the instrument as a clinical tool. Typically, a single condition can be tested for one eye in less than 1 min. Total run time when no runs are rejected, either automatically by the software or by inspection of the raw data displayed on the screen, is approximately 16 s. Thus, once a critical condition is selected for use in the instrument, and no experimental conditions are included in the session, the two eyes can be tested in about 2 min following application of the three electrodes.

One key advantage of the proposed instrument over the commercially available FDT device is that it is a direct measure of physiological activity in the human visual system, and therefore, it does not rely on a psychophysical-linking hypothesis to infer the integrity of the high contrast sensitivity, high temporal frequency (magnocellular) pathway. Although the icVEP test does not assess peripheral visual function, its high accuracy in the classification of glaucoma patients and controls demonstrates the early involvement of central visual function in the disease process. The lack of significant correlations between the icVEP signal-to-noise measures and mean deviation scores obtained with visual field testing provide additional evidence that these techniques tap independent (central versus peripheral) mechanisms.

An advantage of this electrophysiological technique over the multifocal VEP one is its much shorter duration, which results in lower attentional demands. The multifocal VEP, however, has the advantage of providing topographical information over an area equivalent to 24-2 HVF, and therefore, may be of value in monitoring progression of glaucomatous damage. It should also be noted that, unlike psychophysical measures which may indicate normal visual function provided there are some intact neurons sampling a given region of the visual field, the icVEP is a population measure that is likely to reflect a deficit in function relative to the extent of glaucomatous damage.

In the past few years, instruments have been introduced to the field to improve assessment of structural damage associated with glaucoma, and it has been suggested that a combination of structural and functional measures could form the basis of a screening program for glaucoma in the targeted population [36]. Further research is needed to determine if the icVEP may serve as the functional test to be included in the battery of a screening program.

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