Simultaneous Comparison of Relative Damage to Chromatic Pathways in Ocular Hypertension and Glaucoma: Correlation with Clinical Measures

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PURPOSE. To use a new methodological approach, based on luminance noise, to assess without bias the relative damage of blue-yellow and red-green pathways in ocular hypertension and glaucoma and to correlate obtained measurements with clinical markers of disease progression.

METHODS. A psychophysical procedure modified from Cambridge color test was used to assess color discrimination in three different groups: patients with primary open-angle glaucoma (n = 51 eyes), patients with ocular hypertension (n = 95 eyes), and control subjects (n = 46 eyes). Viewing conditions were such that the function of the macula was being tested, using a discrimination task under noisy conditions. Confusion vectors, and parameters obtained from discrimination ellipses were correlated with perimetric and clinical data taken from the same groups.

RESULTS. The lengths of the major axis of chromatic discrimination ellipses and all confusion vectors were significantly different between the groups. These measures followed a significant gradient of worsening performance from the control to hypertensive and glaucoma groups, which was steeper for the tritan axis. There was a significant global positive correlation between test parameters and cup-to-disc ratio and a significant gradient of worsening performance from the control to hypertensive and glaucoma groups, which was steeper for the tritan axis. There was a significant global positive correlation between test parameters and cup-to-disc ratio and a significant negative correlation with the perimetric mean deviation index. Ellipse length in patients with ocular hypertension correlated significantly with the duration of their hypertensive state.

CONCLUSIONS. This psychophysical test can detect visual dysfunction in a significant subset of patients with long-term hypertension with preserved visual fields. Macular function is damaged earlier than previously believed, in both the blue-yellow and red-green pathways. (Invest Ophthalmol Vis Sci. 2004;45:499–505) DOI:10.1167/iovs.03-0815

There is a current controversy in glaucoma research on the degree of relative damage across pathways, in particular regarding the parvo- and koniocellular (more recently identified and which processes blue-on signals1–5) systems. The main reason lies on the difficulty in establishing the best method to promote equivalent activation of different pathways (see Pearson et al.5) and is critical to the evaluation of loss of redundancy and pathophysiologic hypotheses regarding preferential damage. In the current study, we adopted a new methodological approach (testing with randomly interleaved multiple staircases with spatial and luminance noise) that allows independent assessment and in a nonbiased way of the relative damage of blue-yellow and red-green pathways. In addition, we have searched for new psychophysical-clinical correlations, both in early and late disease stages. Appropriate spatial and temporal parameters of stimulation that are not biased toward the function of a single stream, allow investigation of whether the pathway that is affected earliest is also the most affected as the disease progresses. Ganglion cell death is an important feature of glaucoma,4–6 and it is therefore critical to assess how early each pathway is affected and to measure differential damage across different disease stages.

Regarding the specificity of damage within chromatic pathways, it is widely believed that glaucoma is predominantly associated with tritan-like defects.5–7 Because photoreceptors seem not to be significantly damaged in glaucoma,43 most studies imply a predominant involvement of the koniocellular pathway although important parvocellular dysfunction has also been reported.16,20,28,32,54,44 Few studies that try to compare the function of multiple pathways in early and late stages of glaucoma are available, however. Some studies including patients with ocular hypertension have emphasized the level of dysfunction at the visual periphery for the tritan axis but their results have not always been concordant.35,41 Substantial damage has been extensively reported for the magnocellular system45–48 (for a review see Shabana et al.49), partly from indirect evidence that large fibers are preferentially affected early on in this disease.49–51 These findings have formed the basis for the so-called preferential damage hypothesis. There is, however, increased awareness that detectable impairment in a visual pathway depends on its internal degree of redundancy (e.g., the amount of overlap between the receptive fields of its neurons). This hypothesis postulates that damage can be detected only if the degree of redundancy is not too high.3,22,26,53 Effective redundancy may be influenced by effects such as stimulus size in patients with glaucoma. It is also known that the same stimulus size activates different numbers of midget, parasol, and bistratified cells at a given eccentricity, which creates further complications in assessing relative damage.3 The relative level of background luminance may also bias the relative adaptation state of different pathways. These facts may explain the wide discrepancies that can be found in the literature.

Unfortunately, primate models of glaucoma can only partially contribute to solve these issues, because IOP is artificially
P < 0.0001 both for post hoc comparisons between the hypertension and glaucoma groups and between the control and glaucoma groups; P = 0.0045, for comparisons between control and hypertension groups. (C) The length of the discrimination ellipses was significantly increased in glaucoma (P < 0.0001 as a whole, with P < 0.0001 both for post hoc comparisons between hypertension and glaucoma groups and between control and glaucoma groups; P = 0.0578 for comparisons between control and hypertension groups). All vector units are in CIE 1976 u′, v′ color space coordinates, in all figures. Note that in some cases variability was so low that the SE bars are barely visible. The same applies to subsequent figures.

Elevated to very high levels that tend to be associated with more advanced stages of glaucoma. Early functional impairment of small bistratified cells is consistent with the well-documented advantage of blue-on-yellow over standard white-on-white perimetry.19,21–23,26,29,30,35,36 but the specificity of this effect remains questionable.

To compare relative deficits, it is crucial that comparisons be made in the same patient and if possible that the tests be applied in an interleaved manner, simultaneously. We have followed this strategy to evaluate the degree of differential impairment of parvo- and koniocellular function in patients with ocular hypertension or glaucoma, compared with a population of normal age-matched subjects. Stimulus parameters were adjusted to separate and isolate dysfunction within both systems. Size and luminance noise ensured that the measurement conditions were not biased for the activation of any functional stream. Viewing conditions were particularly suited to test the function of the central retina.

This design allowed good activation of both parvo- and koniocellular pathways and made it possible to investigate evidence for early and late concomitant damage. We have found evidence that both systems are affected already at the macular level in ocular hypertension. Earlier studies could not bring much insight into the question, because some did not include subjects with ocular hypertension, and others have only described in detail changes along the tritan axis or have included patient populations that were not age matched. Moreover, strategies based on semiquantitative testing have often been fairly unsuccessful in finding color vision abnormalities in glaucoma. This has been emphasized by Falcone-Reis et al.17,18 and Yu et al.20 who have championed the use of computerized color tests, as an advantage over more traditional semiquantitative methods.

**METHODS**

**Patient Selection and Classification**

A complete ophthalmic examination was performed in all individuals by two ophthalmologists (PF, HA). Control subjects were patients' spouses, age-matched hospital or university staff, or relatives, with normal or corrected to normal refraction. Similar to the other groups, they all underwent a full ophthalmic examination, and no patients with problems unrelated to vision were included. This examination consisted of best corrected visual acuity (VA; Snellen chart), IOP measurement (Goldmann applanation tonometer), slit lamp examination of anterior chamber, angle, and fundus examination (Goldmann lens). Multiple perimetric examinations with Humphrey 50-2 (FASTPAC strategy; Carl Zeiss Meditec, Dublin, CA) were also performed in all groups. The individuals were divided into three different groups: patients with primary open-angle glaucoma (POAG; n = 51 eyes) or ocular hypertension (OH; n = 95 eyes) and control subjects (n = 46 eyes). Patients with POAG filled the following criteria: cup-to-disc (C/D) vertical diameter of 0.4 or more, a mean deviation (MD) visual field global index less than −2 dB (or <5% of confidence interval). Patients with OH were selected according to the following criteria: IOP of 21 mm Hg or more (on at least two occasions), MD more than −2 dB (or >5%, of confidence interval) and C/D less than 0.5. Individuals in the control group had IOP less than 21 mm Hg, C/D less than 0.5, and normal visual fields.

Exclusion criteria included the following: age (<21 or >80), pseudopithak and aphakic eyes, medium significant opacification (conical leucoma or cataract), retinal diseases, neuro-ophthalmic disease, known color vision disorders, VA less than 0.6, and high ametropia (sphere dipters >4 and cylinder dippers >2).

Informed consent was obtained from all participants, and the study was conducted in accordance with the tenets of the Declaration of Helsinki. Our patient and control groups were age matched (control subjects: mean, 57.022 ± 7.603 SD; ocular hypertension, 59.862 ± 7.283 SD; glaucoma, 59.875 ± 9.849). ANOVA showed no significant age difference between groups. There was no sex-related significant difference among the groups for any of the psychophysical measures used. There was no difference in hypertensive medical treatment guidelines across glaucoma and ocular hypertension groups.

**Psychophysical Methods and Analysis**

Viewing conditions were such that all regions the subjects had to consider to perform chromatic comparisons were in the macular region of the retina. Given the importance of size and luminance noise in making sure that the subjects used only chromatic information to perform the test, we used a computer-controlled psychophysical method developed by Regan et al.54 and taken from the Cambridge Color Test (Cambridge Research Systems [CRS], Rochester, UK).

Given the subjects' average age and to exclude confounding factors such as motor errors, subjects performed an oral response, which was converted into a button press response by the experimenter himself (MCB or VF). For further emphasis of accuracy versus speed in the measurement of psychophysical responses, subjects were instructed that they had up to 20 seconds to report their decisions. Subjects viewed monocularly, with refraction corrected for viewing distance, a screen with a pattern of circles of various sizes and luminance with superimposed chromatic contrast defining a gap in a Landolt-like C-shaped ring (Fig. 1A; viewing distance: 1.8 m, gap size: 1.6°, outer diameter: 7.6° inner diameter: 3.81°). The chromaticity of the Landolt C shape was adjusted according to a staircase procedure (see below). The subject had to indicate one of four possible positions of the gap of the Landolt C. Tinted contact lenses and spectacle lenses were replaced by trial lenses in a trial frame. Gap position (bottom, top, left, right) had to be identified, and the experimenter entered the subjects' oral response into a four-button response box. Luminance and size variation of stimulus patches (Fig. 1A) forced the subject to use specific color cues, because he or she could not use spatial or luminance cues (between 8 and 18 cd/m²) to infer the embedded shape. These
patches were randomly assigned six different luminance noise levels (8 to 10 to 12 to 14 to 16 to 18 cd/m²; see also Fig. 1A, for an illustration of the patches). A minimum excursion of 0.002 units was then superimposed on such noise levels, to define the chromatic shape.

Quantitative adjustment in terms of modulation of chromatic contrast allowed for isolation of cone or color opponent-specific responses in Commission Internationale de l’Éclairage (CIE) 1976 u′, v′ color space. Implementation and calibration procedures were performed with software and hardware provided by Cambridge Research Systems: (colorimeter, Minolta, Osaka, Japan; calibration software and CRS/VSG 2/5 graphics card, with 15-bit contrast resolution per pixel; Cambridge Research Systems). Stimuli were displayed on a 21-in. monitor (GDM-F520; Sony, Tokyo, Japan) that was gamma corrected using CRS software and hardware.

Psychophysical thresholds were obtained with algorithms implementing three parallel staircases, from the trivecotor version of the test (which assessed simultaneously the three main confusion axes in color space). This ensures unbiased measurement of thresholds across different chromatic mechanisms. To determine discrimination ellipses, eight confusion line vectors were measured in an interleaved random manner with independent staircases running at a neutral background: neutral point coordinates (u′, v′ coordinates are shown, respectively): 0.1977, 0.4689—minimum excursion: 0.002 units in this space; protan confusion (copunctal) point: 0.678, 0.501; deutan confusion (copunctal) point: −1.217, 0.782; and tritan confusion (copunctal) point: 0.257, 0.0. The maximum excursion for the trivecctor test was 0.1100 (Fig. 2B). Axes angles for ellipses are shown in Figure 6.

Our four alternative spatial forced-choice (4AFC) staircases were interleaved in a random manner to make sure that all color axes were tested simultaneously, which made comparisons regarding relative damage of chromatic pathways very reliable. On each axis, the separation between the background and target chromaticities is initially large and is decreased after each correct response on that axis and increased after each error. The test terminated after 11 reversals of each of the three individual staircases, and the mean of the last 7 reversals was taken as the threshold estimate for a given confusion line. The step size was computed in units of the CIE u′, v′ uniform chromaticity space and is a function of the number of reversals completed and of the separation of test and background chromaticities. A small subset of trials, randomly intermixed with the test trials, were used as control trials to detect malingering and to provide the subject clear feedback regarding the correctness of each response.

The ellipse fitting method we used⁹ produces an ellipse that is centered on the field point and is obtained by minimizing the sum of squares of the log distances between the ellipse and the fitted point, which is a geometric solution for producing discrimination ellipses. We tested this method further, using our own fitting procedure, which consisted of an equiaugmented spline interpolation of the data points around the field point with the determination of the longest diameter (major confusion axis) of this spline curve and a subsequent comparison of the standard deviations of the data points parallel and perpendicular to this axis. We have extracted the following quantitative parameters from the color test results: confusion lines length, ellipse length, and axis ratio. Further statistical analyses (factorial and repeated measures ANOVA, with the post hoc Fisher protected least-significant difference [PLSD] correction; multiple regression) were performed on computer (Statview; SAS, Cary, NC).

RESULTS

Psychophysical Results

Under our testing conditions, robust evidence was found for early chromatic dysfunction in glaucoma. Figure 1B shows a significant and global increase in mean length of the three main confusion vectors (the trivecctor test), as revealed by repeated-measures ANOVA. This increase is significant across all study groups (P < 0.0001 as a whole; with P < 0.0001 both for post hoc comparisons between patients with hypertension and those with glaucoma and between control subjects and patients with glaucoma; P = 0.0045, for comparisons between normal control subjects and patients with hypertension). Figure 1C shows independent evidence of the same finding, through the measurement of discrimination ellipses obtained from the three groups. The ellipse axis length was significantly increased across groups (P < 0.0001 as a whole, with P < 0.0001 both for post hoc comparisons between patients with hypertension and those with glaucoma and between the control and glaucoma groups; P = 0.0578 for comparisons between normal control subjects and patients with hypertension). These values suggest that color confusion vectors are better measures than the length of discrimination ellipses in differentiating control subjects from patients with hypertension, but care must be taken in this interpretation, because ellipses were fitted in fewer eyes (n = 91).

Figure 2 shows a separate analysis of changes in confusion vector length across stages for protan, deutan, and tritan axes. All increased significantly (see legend for details), albeit with a slight tendency for the tritan axis length to show a steeper increase. This global increase across all axes shows that concomitant early damage is already present in patients with hypertension, regarding the konio- and parvocellular systems. The global involvement of chromatic pathways is further suggested by the measurement of axis ratios of discrimination ellipses (Fig. 3). These show a modest increase that can differentiate significance only between control and glaucoma groups.
suggesting that damage may be preferential to the blue-yellow axis but not at all specific. This result becomes more obvious by inspection of Figure 3, which shows that the axis ratio of chromatic discrimination ellipses is less prominently augmented than the elevation of length across stages (Fig. 1C).

We next examined in more detail the question of whether chromatic deficits in glaucoma follow any particular preferred axis. This can best be analyzed by inspection of population distributions of the angles of the discrimination ellipses. Figure 4 shows that the distributions of angles of ellipses were similar (mean, ~80°) in the control, hypertension, or glaucoma groups. In other words, the tendency for worse performance along the blue-yellow axis seen physiologically in normal control subjects was preserved in glaucoma and is evident on inspection of the pie charts in Figure 5. Deviations from this pattern of disadvantage were not seen in ocular hypertension, but only in a small subset of patients with advanced glaucoma.

The observed exaggeration of the physiological disadvantage was subtle but significant. Indeed, repeated-measures ANOVA revealed a significant interaction between groups and type of confusion vector \( (P < 0.0001; \text{see slopes of Fig. 2, showing steeper tritan loss}) \).

**Correlation with Clinical Parameters**

An important goal in this study was to compare our chromatic discrimination measures with important clinical parameters used to classify patients with glaucoma, such as C/D ratio and visual field perimeter measures. Our evidence of chromatic dysfunction in patients with hypertension contrasted quite strikingly with the lack of sensitivity of traditional perimetry measures such as MD. Figure 6 shows representative graphs depicting individual raw data in \( u'v' \) space, as well as fitted ellipses taken from the three study groups. The pattern of progressive deterioration is further documented in Figure 7, which shows percentile distributions of ellipse length. The slight overlap between control and glaucoma groups is remarkable, with the hypertension group showing an intermediate pattern.

A significant deterioration over time of chromatic function was most prominent in patients with hypertension. The deterioration was analyzed by measuring time elapsed since diagnosis and correlating this measure with chromatic performance (length of discrimination ellipses). There was a...
significant correlation only in the group of patients with hypertension ($r = 0.493$, $P = 0.0095$, global $r = 0.206$, not significant).

Regarding the analysis of C/D ratio, this value correlated significantly with almost all our measures of chromatic performance (protan, $r = 0.352$; deutan $r = 0.403$; tritan $r = 0.326$; ellipse length, $r = 0.400$; $P \leq 0.0001$ for all these correlation coefficients taken for the whole population of subjects). The ellipse axis ratio did not show any significant correlation with the C/D ratio, which is not surprising, given the lack of damage specificity that is evident in our data.

Regarding perimetric assessment, we found strong and significant correlations. Indeed, when psychophysical parameters were compared with parameters such as MD, strong global correlations were found (MD 30-2: protan, $r = -0.278$, $P = 0.0009$; deutan, $r = -0.391$ $P < 0.0001$; ellipse length, $r = -0.440$, $P = 0.0015$; axis ratio, ns). Not surprisingly, this correlation is mostly explained by the pattern of loss within the glaucoma group (MD 30-2: protan, $r = -0.545$, $P = 0.0027$; deutan, $r = -0.631$ $P = 0.0001$; ellipse length, $r = -0.479$, $P = 0.0231$; axis ratio, ns). No significant correlation was found with tritan axis length, suggesting that in spite of more prominent tritan loss, this measure is less correlated with field loss, once glaucoma is established.

**DISCUSSION**

The present study shows that a concomitant involvement of multiple chromatic pathways within the central retina is present in the natural history of glaucoma earlier than previously believed. This provides an advance over studies that did not include ocular hypertension, or only described in detail changes along the tritan axis, or have included patients that were not age matched with control subjects. One should, however, emphasize the merit of the work of Falcao-Reis et al. who have championed the use of computerized color tests as advantageous over more traditional semiquantitative methods. Further, we found that progression of chromatic damage correlated significantly with clinical and perimetric measures.

It is worth emphasizing that our computerized approach provides more information than the usual evaluation with the Farnsworth-Munsell 100-hue test, which has been shown to be just a semiquantitative test with bad reproducibility. We used a strategy that allowed for the simultaneous comparison of relative damage of konio- and parvocellular systems, both in

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**Figure 6.** Representative examples of chromatic discrimination ellipses (raw discrimination vectors and fitted ellipses) in individual subjects in the three study groups: (A) control, (B) ocular hypertension, (C) glaucoma. Solid straight lines: measured color axes. Curved solid line: fitted ellipse. Dotted lines: fitted ellipse axes. Color rendering (which is only approximate in the printed version) is based on the IEC1996 2.1 standard, with the white point set to the white point of the test and the monitor gamut set to the gamut of our Trinitron monitor equipped with phosphors. The rendered colors are gamma corrected and the gamut of the monitor is outside the graphs. Parameters extracted from fitted ellipses were as follows (length, axis ratio, and angle, respectively): (A) 0.0116, 1.316, 92°; (B) 0.0203, 2.199, 74.2°; (C) 0.0319, 2.183, 69.9°.

**Figure 7.** Percentile box plots measured for the three study groups. Bars depict 10th and 90th percentiles, the top and bottom borders of the boxes represent the 25th and 75th percentiles, and the line segment inside the boxes depicts the median.
ocular hypertension and glaucoma, as measured by interleaved psychophysical staircase stimuli. These assessed function along the main confusion lines and along multiple color axes that permitted the computation of discrimination ellipses. This strategy may help shed new light on the pathophysiology of glaucoma.

First, we found that significant central damage may have occurred early in a large subset of patients with hypertension, and such damage is not restricted to the blue-yellow pathway, but includes the red-green processing stream. Several reasons may help explain improved detection with our paradigm: size and luminance noise rendered the task particularly difficult and forced the subject to perform chromatic comparisons across multiple locations and between stimuli thus ensuring that the measurement conditions were not biased toward the activation of any functional stream.

It is noteworthy, however, that although early damage was already present in a significant number of patients with hypertension, we also found a subset with no measurable impairment. It is possible that the difference between these subsets is in the amount of time elapsed after diagnosis of ocular hypertension. Many of our patients had been observed for more than 5 years after diagnosis of hypertension, remaining in the pre-perimetric stage. In agreement with our hypothesis, elapsed time since diagnosis correlated significantly with chromatic performance (length of discrimination ellipses in our group of patients with hypertension). Although an age-related confound could be argued, this effect was not significant in our patients with glaucoma.

Our findings of early dysfunction of both parvo- and konio-cellular systems in glaucoma favors the functional-redundancy hypothesis in comparison to the preferential-loss model. It is true that blue-yellow defects predominated in our patients and were actually significantly enhanced in comparison to the normal physiologic elongation, but this effect was of small magnitude. Our careful exclusion criteria avoided other possible causes of significant blue-yellow deficits in our population. That ellipse angle suggested this type of predominant loss should not obscure the significant early proton and deutan losses. It is even possible that such measures may be clinically as relevant as tritan loss. Indeed, they were significantly correlated with the C/D ratio. Furthermore, these measures proved to be significantly good predictors of perimetric damage (along with measures of ellipse length), which is in agreement with a recent finding that development of glaucoma may actually be best predicted by a change in the length of the proton discrimination axis.

We may indeed be facing the problem that a parameter that causes the severest damage does not imply that it is the most useful measure, because no significant correlation was found between tritan axis length and perimetric damage under the conditions of our measurements. Despite the prominent tritan loss, this measure correlated the least with progression of field loss once glaucoma was established. Further studies are necessary to clarify the relation between predominant damage and field loss as considered in traditional assessment procedures and predominant progression and field loss. Significant damage to the red-green chromatic pathway has been associated only with advanced glaucoma. Our data extend these results to ocular hypertension, in that and show that in a small subset of patients with advanced glaucoma, a predominant red-green loss may also be found.

We favor the view that assessing early macular involvement may provide clinically reliable and reproducible test measures, given that the amount of overlap across populations was much reduced in comparison with earlier studies. This trend has been observed by some research groups: Adams et al. and Heron et al. using a flicker detection method, suggested that foveal chromatic function may be perturbed relatively early on in the disease process, although mostly through the short-wavelength sensitive pathway. This question was reexamined by Greenstein et al., who discriminated of a 5° disc from a white background, who found losses among both opponent systems. Pacheco-Cutillas et al. who also focused more on the fovea than on the whole of the macula (we prefer the latter approach), found similar results, although no significant conclusions are available so far on groups of patients with ocular hypertension.

Future studies should help define psychophysical conditions that help to improve the strategy used in this study for early detection of functional damage induced by high IOP. Taking advantage of static size and luminance noise helps the isolation of multiple chromatic pathways in an unbiased manner, but it remains to be clarified whether dynamic spatiotemporal noise will further improve this goal. We favor static noise, because temporal changes may cause unpredictable activations in the magnocellular system.

In summary, our findings suggest that earlier detection of functional damage can be achieved in both the red-green and blue-yellow chromatic pathways than previously believed. Optimized psychophysical procedures based on luminance noise may allow for the establishment of new clinical correlations that are useful to quantify disease progression and to define subpopulations of patients with ocular hypertension that may evolve into glaucoma.

Acknowledgments

The authors thank Adozinda Simões for help in patient selection.

References


