

PERFORMANCE OF NONLINEAR VISUAL UNITS IN OCULAR HYPERTENSION AND GLAUCOMA

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(Received 22 August 1991; in revised form 7 January 1992)

Summary-I. Two psychophysical tests to assess retinal function in ocular hypertension and glaucoma are described. The basis for these tests is the frequency-doubled percept seen with rapid temporal modulation of the contrast of very low spatial frequencies.

2. The results of the second set of experiments show correlation ($P < 0.01$) with optic disc cupping. Without the inclusion of information about subjects' optic disc condition the second experimental procedure distinguishes the test group from normals ($P < 0.01$).

3. A model which considers optic disc condition in conjunction with the results of the main experimental method described leads to a separability of normals and a subgroup with moderate disc cupping at the $P < 0.001$ level.

4. The results reported here, taken together with the current data on retinal ganglion cell function, indicate that harnessing unique features of the frequency-doubled illusion may allow the design of a highly specific and sensitive test for the assessment of early glaucomatous damage.

Key words--Glaucoma; psychophysics; magnocellular; M_L cells.

INTRODUCTION

Recent evidence suggests that elevated intra-ocular tension leads to the selective loss of large retinal ganglion cells (Quigley *et al.*, 1987, 1988; Glovinsky *et al.*, 1991) that project to the magnocellular layers of the lateral geniculate nucleus (LGN). In the primate LGN, the two magnocellular layers lie ventral to the parvocellular layers, which in different species may be two or four in number (for a review see Henry, 1991). Most, if not all cells of the parvocellular layers, are colour selective while those of the magnocellular segments are colour insensitive. In the last few years, much more has been discovered about other distinctions in the response properties of ganglion cells projecting to the magno- and parvocellular layers and functional class names for ganglion cells are now based on the cell's destination in the LGN: M cells are those projecting to the magnocellular layers and P cells to the parvocellular layers. While we shall not detail these class differences (for the latest review see Kaplan *et al.*, 1990), it is necessary, for the present study, to appreciate that there is a subgroup of M cells, the M_L cells,

that pool the responses from different parts of the cell's receptive field in a nonlinear manner. In contrast, summation in receptive fields of P cells, and the other subclass of M cells (M_X), is quite linear so that it should be possible, using the nonlinear characteristic of M_X cells as a signature, to develop an exclusive test for the M_L pathway and to use this for the detection of loss of large ganglion cells resulting from maintained or occasional elevation of intraocular tension.

The present study is based on a visual illusion that was first described by Kelly (1966). Kelly (1981) subsequently found that the phenomenon he observed, and described as spatial frequency doubling, resulted from the activation of a nonlinear pathway which, in the light of the recent differentiation of M and P cells, may correspond to the " M_L " pathway. Kelly, in the original experiments, found that when coarse gratings, made up of light and dark bars with the light intensity profile of a sine wave, had their contrast reversed at a relatively high rate, the number of bars (cycles) in the grating appeared to be doubled. Two parameters have an influence on whether the grating appears to double, the first is the spatial frequency of the grating and the second is the

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temporal frequency at which its contrast is modulated. Depending on the retinal eccentricity (Maddess, 1992) the spatial frequency should be between 0.1 and 4 c deg⁻¹ and the temporal frequency greater than 15 Hz. In this visual illusion the rapidly-flickering gratings are seen to be relatively stable but with twice as many cycles. The doubling is now thought to arise from second harmonic distortion in the rapidly responding nonlinear visual pathway which we refer to as the "M_Y" pathway.

In laboratory experiments recording the responses of single cells in the LGN of macaque monkeys between 5 and 20% of the cells in the magnocellular layers are found to respond with a nonlinear or Y type of response that is dominated by second harmonic distortion (Kaplan and Shapley, 1982; Marrocco *et al.*, 1982; Derington and Lennie, 1984; Blakemore and Vital-Duran, 1986). The remainder of the LGN cells have linear responses and little or no second harmonic distortion. Recordings from the presynaptic retinal ganglions indicate that LGN cell responses accurately reflect the retinal ganglion cell responses (Kaplan and Shapley, 1986; Crook *et al.*, 1988; Michael, 1988; Purpura *et al.*, 1988, 1990). These findings make the M_Y cells a good prospect as the cell type contributing to Kelly's nonlinear visual pathway and further support comes from psychophysical investigations in our laboratory (Maddess, 1992) which have shown that the cell density of the nonlinear pathway is similar to that of the M_Y system.

It is worth restating that there is now good evidence that larger retinal ganglion cells are more susceptible to glaucomatous damage (Quigley *et al.*, 1987, 1988; Glovinsky *et al.*, 1991). At the same time three single cell electrophysiological studies indicate that M_Y cells are larger than M_X cells (Kaplan and Shapley, 1982; Marrocco *et al.*, 1982; Blakemore and Vital-Duran, 1986) by virtue of their larger receptive field size and more rapid conduction velocities. Also, the coverage factor of M_Y cells is very low (Crook *et al.*, 1988) indicating that the loss of even a single cell will lead to a distinct scotoma in the lattice of M_Y cell receptive fields. Another factor which makes the frequency-doubled illusion and M_Y cells interesting from the perspective of glaucoma diagnosis is that other neurological disorders which commonly produce visual deficits do not affect vision in the spatiotemporal region requisite for seeing the frequency-doubling illusion. For example am-

blyopia (Hess *et al.*, 1978), optic neuritis (Hess and Plant, 1983; Edgar *et al.*, 1990) and Parkinson's disease (Marx *et al.*, 1986) do not appear to affect perception of rapidly flickered coarse patterns. Thus, stimuli tailored to production of the frequency-doubled illusion may stimulate a visual pathway highly sensitive to glaucomatous damage and the results of any test for glaucoma based on such stimuli may be little affected by other neurological disorders affecting vision.

The present study, therefore, set out to adapt the frequency-doubled illusion to find a way of assessing the functional effectiveness of the M_Y pathway. The experiments were designed to compare the performance of the nonlinear pathway in samples from two populations of human subjects, one where there was no history of elevated intraocular tension and the other where there was a clinical record of an elevated tension that may have damaged the M_Y system at some time in the past. If it proved possible to differentiate individuals in the two samples then the test would be assessed to discover how well it could be applied to the clinical situation and how well it could be related to the other manifestations of glaucoma.

METHODS

Experimental subjects

The subjects were placed in one of two sample groups. The first, or control group, consisted of 17 individuals with no clinical history of elevated intraocular tension and no signs of ocular pathology. The second or test group, made up of patients from private ophthalmological practices, contained 27 persons, all with a clinical record of intraocular tensions of 20 mm Hg or more. Two of the test group, with intraocular tensions that had only reached 21 mm Hg, were diagnosed as having as normal tension glaucoma, since they both had arcuate scotomas characteristic of glaucoma. Three other subjects in the test group had arcuate scotomas in at least one eye. In all 18 of the 27 test group subjects were using topically applied timolol and, at the time of the experiment, the mean intraocular tension for the 27 test subjects was 18.5 mm Hg \pm 5.27 (SD) which had been reduced with the medication from a highest-reading mean of 26.2 mm Hg \pm 5.89 (SD). The recorded tensions of 15 members of the test group had not exceeded 26 mm Hg and for 7 others the highest reading had not exceeded 23 mm Hg.

The optic discs of both control and test subjects were assessed and ranked into one of four classes on the basis of the relative extent of optic disc cupping (vertical cup to disc ratio). These classes were used in the statistical analysis of the results but it should be emphasised that we have not attempted a precise grading of cupping but rather to create two divisions inside the two extremes of zero and total cupping. The broadness of the grouping is likely to increase the variance within each class and is a statistically conservative move. Thus, if there was little or no cupping the subject was placed in class 1; if the ratio of vertical dimension of the cup to that of the disc was in the region 0.25–0.50 the subject was placed in class 2; if between 0.50 and 0.8, in class 3 and if the cupping was total, in class 4. Two control subjects with congenital cupping were placed in class 3 although neither had displayed intraocular tensions above 17 mm Hg nor showed any signs of visual field loss. All other members of the control group were in class 1.

Visual display

Visual images were presented on the cathode ray tube (CRT) of a Joyce Visual Display Unit, which had a white (P4) phosphor and a mean luminance was adjusted to 297 cd m⁻². The CRT's response was linear over the range used in the experiments and only became nonlinear to the extent of a few percent at high contrasts above 70%. Spatial modulation and contrast level were controlled by cascaded digital-to-analogue converters which enabled the spatial resolution of the waveforms of up to 256 points per cycle, and minimal displacements of 1/256th of a cycle, and 4096 levels of contrast per waveform. Temporal modulation followed a sine wave time-course defined by a single cycle lookup table of 1000 entries. The image refresh rate or frame rate of the CRT was 100 Hz or every 10 ms. At the viewing distance of 72 cm the display was 12.8° high and 16° wide. Subjects viewed the display monocularly while their heads rested against chin and forehead supports.

Experimental procedures

The primary object of the experiment was to find a procedure that would effectively detect reductions in the performance of the M_L pathway but at the same time would be quick and easy to implement in the clinical setting. To fulfil this aim we used two experimental paradigms

for both groups of subjects, who were fully apprised of all procedures and so were able to give informed consent.

Method 1-movement used in the measurement of contrast threshold for a frequency-doubled grating. The subject's task in this experiment was to determine the direction of movement of a test grating for which the contrast was lowered by 20% with each correct response. For incorrect responses the contrast was doubled and the contrast associated with this "reversal" was recorded as a threshold reading. Each pattern was presented 26 times to obtain an average of about 6 reversals/pattern. Experience indicated that this produced a limen of about 60%. The test pattern was set to drift either to the left or the right at 3.13 deg s⁻¹, while its contrast was modulated with a sine wave time-course at the following rates: 0, 23, 28.5, 34, 39.5 and 45 Hz. The thresholds for the 6 modulation frequencies were established in parallel with the various modulation frequencies presented in randomised order. In the terms used in experimental psychology this procedure is described as a multiple-staircase, two-alternative, forced-choice method. The test gratings, selected to produce a strong frequency-doubled effect, had a spatial frequency of 0.25 c deg⁻¹ (the most effective frequency recorded by Kelly (1966) for the given display size (Maddess, 1992)), and the pattern intensity was modulated horizontally (i.e. vertical bars).

Of the 6 patterns described above the grating whose temporal contrast was not modulated had a different spatial frequency 0.5 c deg⁻¹ which allowed its spatial appearance to be similar to the frequency doubled gratings. Subjects were allowed to inspect the patterns for up to 30 s but more often than not they made decisions about the pattern's direction of drift within 1-2 s. These long inspection periods were employed to counteract the effects of temporal summation which affects thresholds for stimuli like those used here (Hess and Howell, 1988). The large size of the patterns also minimised the effects of spatial summation (Hess and Howell, 1988).

Method 2-form used in the measurement of contrast threshold of a frequency-doubled grating. In the second experimental procedure, subjects were asked to find, by the method of adjustment, the threshold of visibility of a frequency-double pattern produced by a stationary 0.25 c deg⁻¹ grating, sinusoidally modulated at frequencies of 12, 17.5, 23, 28.5, 34, 39.5 and

45 Hz. Preliminary tests showed that as the contrast was reduced a point was reached where the frequency-doubled pattern disappeared and was replaced by a faint amorphous flicker, which in some cases persisted over a small range of reduced contrasts until finally the display was seen to become quiescent. It was stressed to the subjects that the threshold point occurred at the point when the vertical grating pattern was replaced by the sensation of amorphous flicker. At contrasts close to threshold, it was also noted that steady fixation often led to the disappearance of the grating around the fixation point. Subjects were therefore asked to reduce the contrast until the pattern was "just not visible at any point on the screen" and to allow their gaze to rove naturally about the display. This condition was thought to be the one more likely to occur in the clinical situation. In making measurements the subjects were required to operate five keys on a computer keyboard and the experiment was repeated 4 or 6 times although in all cases the replicated readings were not significantly different ($P > 0.05$).

Statistical analysis

Statistical analysis was conducted with the aid of the Genstat Analysis Package and Dobson (1983) should be consulted for an introduction to the use of this package and for a list of similar packages. Examination of the threshold data obtained from both methods showed that the measured variances were heterogeneous and that a log transform was indicated for subsequent analysis. When the log transform was applied the resulting data and the residuals from fitted models both showed normally distributed variances. This and other standard diagnostics indicated that a log transform was justified. Similar transformation, or examination for its need, has not always been followed in the psychophysical assessment of glaucomatous damage performed in other laboratories, although some have tacitly employed a log transform by presenting their threshold data in decibels, a logarithmic unit. We mention this because the differential treatment of such psychophysical data in the literature makes comparison more difficult.

RESULTS

The results from the two methods were subjected to an analysis of variance to test

the significance of a number of sample parameters. No significant age or sex effects were found in the results from test or control groups using the two experimental methods. The ages of the control and test groups were 47.9 ± 10.5 (SD) and 53.9 ± 13.5 (SD) yr, respectively.

Method 1-detection of movement of frequency-doubled grating

Method 1 was used to determine the contrast threshold to detect the direction of movement of a horizontally drifting vertical grating (0.25 c deg^{-1} which was flickered at a range of frequencies to create a frequency-doubled appearance. Five temporally modulated and one unmodulated grating were presented in a randomly interleaved 2AFC procedure (Methods). The concept behind including one unmodulated grating was to provide some basis for comparison between conventional contrast thresholds and that obtained with frequency-doubled conditions. The procedure was however complicated by the use of the direction of motion as the cue for establishing the threshold.

Since we were concerned with making a comparison between the thresholds obtained for temporally modulated and unmodulated gratings we first submitted the six thresholds from each subject to a principal component analysis. The first two principal components were indeed primarily correlated and composed of either the five modulated gratings or the unmodulated grating. Of these two orthogonal components the one which was basically a mean of the modulated frequencies was a significant determinant of whether a subject was in the control or the test group ($P < 0.05$). In contrast, with the principal component primarily associated with the unmodulated (0 Hz) grating there was no significant difference in the mean contrast thresholds measured in the two groups of subjects.

For purposes of comparison with method 2 the two principal components were then subjected to a canonical variate analysis (or discriminant analysis). The false positive rate was 43%. That is to say using information derived from the mean threshold for the frequency doubled stimuli about 40% of normals would be diagnosed as glaucomas. The false negative rate was 27%. The data from the 0 Hz grating was less valuable being rejected by the canonical variate analysis.

Method 2-visibility of frequency-doubled grating

In the second series of experiments the contrast threshold for seeing the frequency-doubled pattern was obtained by using a "method of adjustment" procedure to make a direct assessment of whether the form of the grating was visible. This method had the advantage of being relatively simple and so would be useful in the clinical setting. Another aspect of the test, of possible clinical advantage, was that the measurements were highly repeatable; thus the variance in the responses of individual subjects was small, being about 10% of the mean threshold for any particular test condition. Subjects were routinely questioned about what they saw at threshold and they all claimed to be able to see the same number of grating cycles as at higher contrast. The observation of frequency-doubling at threshold is expected given that the purported non-linearity, rectification, is "hard" operating even at low contrasts.

Figure 1(a) shows the differences between the mean thresholds for test and control groups. The elevation of the thresholds in the test group was more marked than in the results from method 1. The mean thresholds for the test group are shown in the upper (dashed) curve in Fig. 1(a) while those for the control group are joined by the lower (dotted) curve. Figure 1(b) expresses the difference in the thresholds for the two groups as a ratio and shows that, at all flash rates, the mean thresholds for the test group were about twice those for the control group. Figure 1(a) also includes error bars representing the standard deviation (left facing) for the threshold measurement at 39.5 Hz and both Fig. 1(a) and (b) contain the 95% confidence limits (right facing error bars) for all threshold points. The variances of the threshold measurements grew as the thresholds increased at higher temporal or flash frequencies. This lack of variance homogeneity was also accompanied by skewed distributions around each mean value and these two features prompted the application of a log transform to the data (Methods).

The log_e transformed data are shown in Fig. 2 and the standard deviation bars (left facing) and confidence limit bars (right facing) are now relatively constant over the full range of frequencies. The difference between the two sets of measurements was significant ($P < 0.01$) and the standard deviations only come close to overlapping at two frequencies, 28.5 and 34Hz. Note that the difference in the mean thresholds, at all flash frequencies, is now close to equal as reflected in the threshold ratios in Fig. 1(b).

The spread in the distribution of thresholds for each group in Fig. 2 was largely due to variation between individuals rather than within the threshold measurements of any particular individual. For example, as mentioned above, the standard deviation for the threshold measurements of an individual was about 10% of that individual's mean threshold value and on average the difference between the mean thresholds for the two groups represents slightly more than six individual standard deviations. This relationship is similar to one reported in earlier experiments (Tyler, 1981) where the contrast of a 2° spot, a stimulus dominated by low spatial frequencies, was modulated at flicker rates comparable to those used here. It is possible, therefore, that this stimulus arrangement tested for the same deficits as the frequency-doubled stimulus and likewise assessed

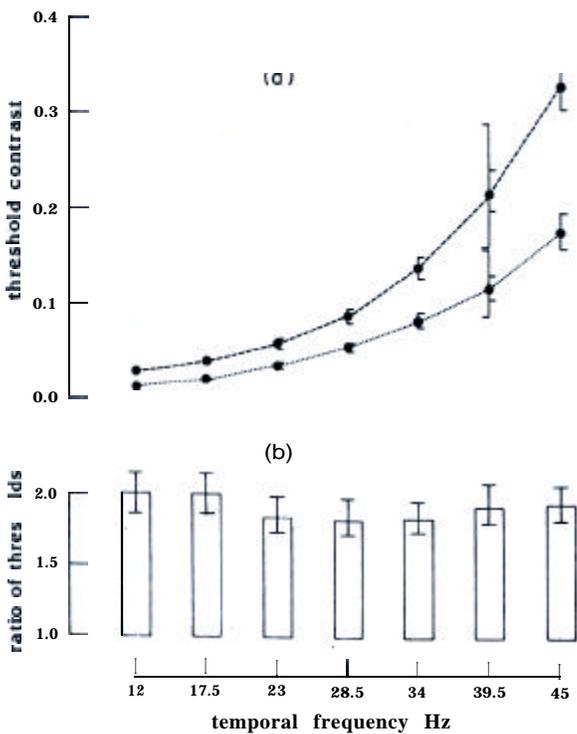


Fig. 1. (a) Contrast thresholds for the test group as a function of temporal modulation frequency where 1.0 equals 100% contrast. The points joined by the dashed line are results for the test group and those joined by the dotted curve are results for the normals. The large, leftward facing error bars are population standard deviations and the smaller rightward facing error bars are 95% confidence limits. Notice that the variance of the thresholds scales with the mean threshold values. (b) The ratio of the test/normal group threshold presented with 95% confidence limits.

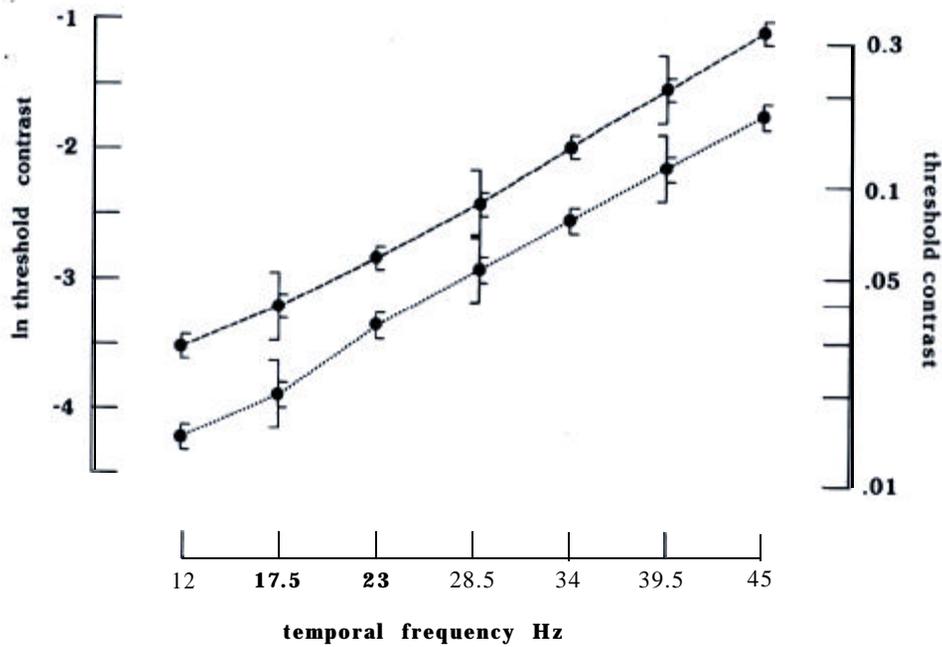


Fig. 2. Thresholds presented as the natural logarithms for the test (dashed upper curve) and normal (dotted lower curve) groups. The groups are different at the $P < 0.01$ level. The left ordinate shows the equivalent absolute contrast for ease of comparison. Note that the variances of the thresholds is now independent of the mean threshold values. Conventions for error bars are as for Fig. 1.

the performance of M_L cells. It should be group then the difference between the control pointed out that had we used the untransformed and test groups would be equivalent to about standard deviation of individuals in the control 25, instead of 6, individual standard deviations.

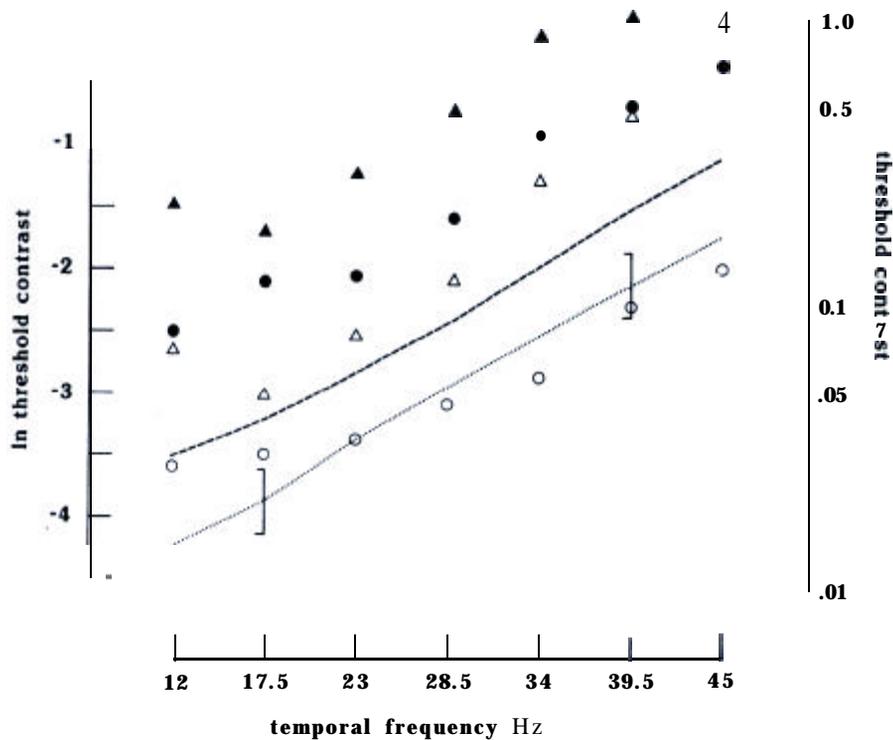


Fig. 3. Visual performance of two test subjects, DFG (open symbols) and MKL (solid symbols) who had glaucomatous scotoma in at least one eye. The thresholds in each eye are consistent with the damage levels in their eyes. Thresholds for their left and right eyes are denoted by circles and triangles respectively. Subject MKL had a ring scotoma in the right eye and an arcuate scotoma in the left while DFG had an arcuate scotoma in the right eye and no scotoma in the left. Notice at 34 Hz that MKL's performance is 7 population standard deviations away from normal performance or more than 20 individual standard deviations. The curves of Fig. 2 are provided for reference as are 2 standard deviation error bars.

Threshold measurements as a pointer to retinal damage

Figure 3 has been included to give an indication of how individuals perform in making threshold measurements and to see if these measurements can be related to the degree of retinal damage. In other words to obtain an indication if between subject variance was due more to individual performance or retinal condition. Two subjects were selected as examples on the basis that they had known differential damage in the two eyes: A, represented by open symbols; and B, by solid symbols; each had visual field losses that had been attributed to glaucoma. The threshold measurements, made for each eye (triangles for right eye and circles for left), were the means of three interleaved trials. The individual standard deviations were about 10% of the mean threshold and in both cases the threshold values in the right eye were significantly higher than those in the left. This difference in the test results appeared to reflect the level of damage as expressed in the perimetric field loss. Thus subject B had an arcuate scotoma in the left eye and a more advanced ring scotoma in the right. The threshold measurements paralleled the field loss differences between the two eyes and the thresholds for the right eye were elevated above those for the left. Subject A, in turn, had an arcuate scotoma in the right eye and no scotoma in the left. Again, the field loss differences are reflected in the threshold measurements and indeed suggest there is little retinal damage in the left eye. The curves from Fig. 2 are included for reference in Fig. 3.

The examples in Fig. 3, therefore, helped to advance the idea that the threshold measurements of individuals could provide a pointer to the level of damage in the M_L pathway. Such a comparison of individual thresholds only has meaning, however, if it can be shown that the variance of the readings from an individual are not just errors in judgement. The fact that the variance of the threshold measurement within individuals is much less than that between individuals in encouraging and suggests that the threshold variation across the group may indicate damage levels. More direct support comes from examples like those in Fig. 3 where large threshold differences, found in each eye, aligned with estimates of retinal damage derived from perimetric field plots.

Threshold measurements and cupping of the optic nerve head

Additional support for the proposal that individual threshold measurements give a guide to the extent of retinal damage comes from their relationship with the appearance of the optic disc. Disc cupping has long been a classical diagnostic indicator for glaucoma (for review see Leske, 1983) and in the present series of experiments subjects were classified into four groups based on the ratio between the vertical diameter of the cup and the nerve head (see Methods). A significant relationship was found between the four cupping classes and the elevation of the threshold in the test group using method 2 ($P < 0.01$). Thus in broad terms the wider the cup the higher the threshold.

To take the analysis further we carried out a regression analysis of the thresholds against the four classes. The outcome of this analysis is presented in Fig. 4 where the four classes (1 to 4) are plotted against sample thresholds presented, in each graph, for one of four different temporal frequencies (12, 23, 34 and 45 Hz). Since the cupping worsens with each class, a regression line of steep positive slope would indicate a strong relationship between cupping and threshold elevation. The slopes are not spectacularly steep in Fig. 4 but, for each frequency, the slope of the regression line was significant (t-test values ranged from 15.71 to 23.69, and the r^2 values from 0.746 to 0.870, d.f. = 84). The significance of this relationship would be reduced by the coarseness of the classing procedure so that our estimate is a conservative one. It is clear, however, that there is considerable overlap in the threshold measurements in each class so that, although members of a higher class are likely to have a higher threshold, their readings do not fall into a segregated range of values. The slopes of the regression lines at different frequencies do not differ significantly from one another although there is a slight bias towards higher slopes at higher temporal frequencies and once again this is in agreement with results obtained by Tyler (1981) with a different but related stimulus.

The evidence that threshold for detecting the frequency-doubled image increases with visual impairment and with disc cupping has led us to consider separate statistical treatment of the thresholds from each cupping group. If, in taking this step, we attend to the practicalities of the present project, that is to discover how

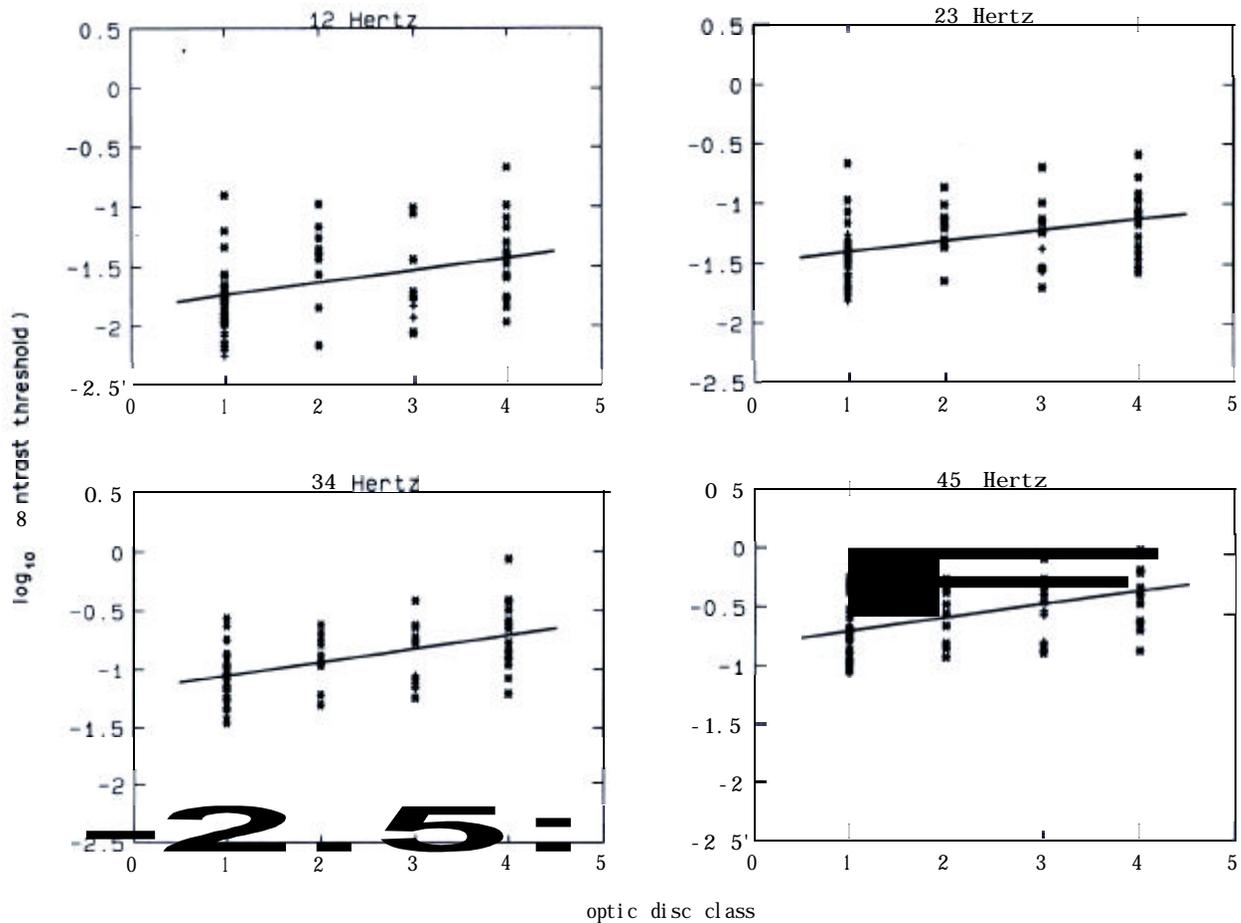


Fig. 4. Regression of \log_e (contrast threshold) vs optic disc class (Methods) for 4 of the 7 temporal frequencies tested. While there was no significant difference in the slopes of the curves at any temporal frequency the disc classes were significant determinants of the thresholds at the $P < 0.01$ level.

effectively threshold elevation acts as a pointer to retinal impairment, then some regrouping of data is possible. For disc class 1 to be treated as the normal or control group then there is a justification to remove the glaucoma suspects from this group. In addition, the two non-glaucomatous subjects in disc class 3 (see Methods) were also excluded because they had elevated thresholds and could not be included with confidence in the control group. With this reorganization, classes 2 and 3, when compared alone, were found to be significantly different ($P < 0.001$) from the control subjects in class 1. This finding was of interest from the clinical point of view since only one of these subjects had a glaucomatous scotoma.

With the thresholds in classes 2, 3 and 4 being so significantly different from those in class 1 it seemed reasonable to test the effectiveness of the threshold measurement for predicting whether or not the subject would fall in class 1. For the purpose of this analysis, therefore, classes 2, 3 and 4 were grouped together as the "model group" and canonical variate analysis was

applied to predict from threshold measurements the numbers likely to be allocated to the control or model groups. These allocations are set out as follows:

Of the persons placed in the control group from the appearance of their discs, 24/28 would be similarly allocated by their contrast threshold measurement. Likewise, of the persons in the model group, 28/34 would be similarly placed by threshold measurement. In comparison with method 1 then the false positive rate was 14% (cf. 43%) and the false negative rate 18% (cf. 27%). The model, with its reliance on elevated threshold readings, is reasonably successful, therefore, in predicting the occurrence of glaucomatous damage.

Threshold measurements and intraocular tension

It was not a straightforward matter to compare the measured contrast thresholds with prevailing intraocular tension since so many members of the test sample were receiving medication to lower intraocular tension. However,

one subject, diagnosed as having low-tension glaucoma by virtue of tensions always measuring below 21 mm Hg and showing an arcuate scotoma, had contrast thresholds well above the control group. All attempts to show a significant correlation, between threshold elevation and prevailing or highest recorded intraocular tension, were unsuccessful but this does not exclude the possibility that threshold elevation is related to an elevated tension that, at some time, has damaged the retina and gone undetected clinically.

DISCUSSION

Conditions associated with an elevated threshold for the detection of frequency-doubled gratings

We will begin by surveying the essential ingredients in this study. A group of subjects comprising persons with elevated, but not excessively high, intraocular tensions (26.1 ± 5.89 mm Hg) and few glaucomatous scotoma, were found to have significantly elevated contrast thresholds for detecting frequency-doubled gratings when compared to a control group. There were indications from binocular differences in individual subjects that the elevation in contrast threshold was related to irreversible neural damage. Consistent with the apparent relationship to neural damage, a correlation was also found to exist between an increase in threshold and a greater cupping of the optic disc. These associations all suggested that the measurement of contrast threshold for the visibility of a frequency-doubled grating provides a good indicator of neural damage from elevated intraocular tension. One significant contra-indicator of the clinical usefulness of the threshold reading appeared in the failure to find any direct correlation with intraocular tension *per se*. This lack of relationship applied both to the intraocular tension measured at the time of the experiment and to the highest clinically recorded reading. This result is not surprising given the abundant evidence that intraocular tension and glaucomatous damage are not very well correlated (for a review see Leske, 1983), and none of our subjects ever had a very high recorded tension (Methods).

Indications that the nonlinearity responsible for frequency doubling occurs in the M_Y pathway

Electrophysiological studies on neurons in the macaque monkey's retina and LGN are

gradually accumulating information about the properties of cells in the M_X pathway (Kaplan and Shapley, 1982; Marrocco *et al.*, 1982; Derrington and Lennie, 1984; Blakemore and Vital-Duran, 1986) and it is now possible to establish a number of features in the stimulus requirements of these cells that are also necessary to invoke the frequency-doubled illusion. Thus the cells of the M system respond well to stimuli of high temporal frequency and low spatial frequency which are the optimum requirements for the production of the illusion. Moreover, behavioural studies involving selective lesions of the P and M layers of the primate LGN (Merigan and Eskin, 1986; Merigan and Maunsell, 1990; Schiller *et al.*, 1990), show that responses to rapidly flickered achromatic stimuli are governed by the M system. The fact that the present series of experiments uses a contrast threshold measurement also increases the likelihood of involving the M rather than the P pathway. This follows the demonstration, in both electrophysiological studies of the single retinal ganglion cells (Kaplan and Shapley, 1986; Crook *et al.*, 1988; Purpura *et al.*, 1988, 1990) and ^{14}C -2-deoxy-*d*-glucose studies (Tootell *et al.*, 1988), that the M retinal ganglion cells have a much higher contrast sensitivity than those of the P pathway. In the nature of the response also there is a similarity in the performance of M_Y cells and the degree of illusion in that both can be described by a similar mathematical model, one that applies to the performance of a compressive power law rectifier: $|x|^P$ where $P < 1$ (cf. Kelly, 1981 with Victor and Shapley, 1979). Also, retinal gain control, originally characterised in cats (Shapley and Victor, 1978) and thought to exist in primates (Derrington and Lennie, 1984), will tend to greatly emphasise the response of M_Y cells over M_X cells at high temporal frequencies. This is because even at contrasts of a few percent the retinal gain control results in a superlinear enhancement of the Y cell response to stimulus frequencies of around 20 Hz. For example Shapley and Victor (1979) report for Y cells a 20-fold response increase to an 8-fold increase in contrast at 15 Hz but only a 3-fold increase in the response to stimulation at 0.5 Hz. By contrast X cell responses grow sublinearly or at best linearly in response to increments of contrast. Thus, there is the potential at frequencies around 20 Hz for the Y cell class to respond much more vigorously than their X cell counterparts. Finally, in a companion study

(Maddess, 1992), using another series of psychophysical experiments, one of us has obtained results to suggest that the density of units driving the frequency-doubled percept matches the expected density of M_Y cells over a wide range of retinal eccentricities.

Virtues of using the M_Y pathway for the early detection of retinal damage

There is increasing evidence that there is diffuse as well as local ganglion cell loss in glaucoma (Quigley *et al.*, 1982; Airaksinen *et al.*, 1986; Schulzer *et al.*, 1987). These diffuse losses are difficult to detect with conventional methods and Quigley *et al.* (1982) have described a subject who, although missing 40% of his retinal ganglion cells, showed no field losses, had no visual acuity loss and had only moderately cupped discs. The frequency-doubled illusion tests the performance of the retinal area covered by the image of the visual display and, therefore, its generation is affected by any loss in this extended area. In addition, the M_Y system may have a greater sensitivity in registering cellular damage than other, more populous systems, such as provided by the P pathway. This greater sensitivity to damage arises from two sources: cell size and retinal coverage factor.

Early suggestions that larger retinal ganglion cells were more susceptible to glaucomatous damage (Quigley *et al.*, 1987, 1988) have now been bolstered by a new study (Glovinsky *et al.*, 1991) indicating that retinal ganglion cell loss in glaucoma is directly proportional to ganglion cell size, the largest cells being lost most rapidly. At least three studies indicate that M_Y cells are larger than M_X cells. Blakemore and Vital-Duran (1986, Fig. 7) show that at a given retinal eccentricity M_Y cells have lower spatial frequency resolution than M_X cells, indicative of larger receptive and so dendritic fields. Larger receptive fields were also reported for M_Y cells by Kaplan and Shapley (1982). Perhaps more direct evidence for larger M_Y cell size comes from studies of conduction latency (Kaplan and Shapley, 1982; Marrocco *et al.*, 1982) which show that M_Y retinal ganglion cells have significantly lower optic chiasm to LGN conduction latencies than M_X cells, indicating larger calibre axons.

The second reason for M_Y cell sensitivity to glaucomatous damage may reside in retinal coverage factors. Recent estimates of the ganglion cell coverage factors (or number of

receptive fields/image point) are about 3.2 for M cells and about 24 for P cells (Crook *et al.*, 1988). Evidence from studies on humans (Maddess, 1992) and macaques (Kaplan and Shapley, 1982; Marrocco *et al.*, 1982; Derrington and Lennie, 1984; Blakemore and Vital-Duran, 1986) indicate that the M_Y cells make up about 10% of the M pathway and as a result their coverage factor would be < 1 ($3.2 * 0.1 = 0.32$). It follows that the performance of the M_Y system would be particularly susceptible to any diffuse cell loss across the retina because there would be little redundancy to hide the loss. Put simply, due to low coverage factor the loss of any M_Y cell will result in a hole in the lattice of M_Y cells, there being no redundant field coverage to hide the resulting scotoma.

Another reason for wishing to study the M_Y pathway involves the issue of specificity for glaucomatous damage. In addition to being sensitive to glaucomatous damage any test for glaucoma should also not provide answers confounded by other neurological disorders. The frequency-doubled illusion occurs at very high temporal frequencies and low spatial frequencies. These are stimulus conditions generally attributed to the transient M pathway: stimuli at the opposite end of the spatiotemporal spectrum being thought of as the domain of the P system. Other common disorders affecting vision, such as amblyopia (Hess *et al.*, 1978), optic neuritis (Hess and Plant, 1983; Edgar *et al.*, 1990) and Parkinson's disease (Marx *et al.*, 1986), appear to primarily suppress the sensation of fine patterns presented at slow temporal frequencies, suggesting dysfunction mainly of the P system. Indeed, in primate (Hendrickson *et al.*, 1987) and human (von Norden *et al.*, 1983) amblyopia it is established that it is mainly the P pathway which is affected. Taken together the above information suggests that specificity for glaucomatous damage is unlikely for temporal frequencies much below 15 Hz or spatial frequencies much above 1 c deg⁻¹.

Other psychophysical experiments designed for the detection of retinal damage from elevated intraocular tension

A number of experiments have been conducted to see if colour vision defects are acquired with the onset of glaucoma (Drance *et al.*, 1981; Adams *et al.*, 1982; Hamill *et al.*, 1984; Airaksinen *et al.*, 1986; Heron *et al.*, 1988). Tests based on the detection of acquired colour vision

defects have been moderately effective in the early detection of glaucoma but, in one sample, as many as 25% of subjects with glaucomatous scotoma showed no colour deficit (Airaksinen *et al.*, 1986) and also colour defects were not found to be correlated with optic disc cupping (Hamill *et al.*, 1984). In line with the interpretation used in the present study, colour vision deficits would indicate a decline in the performance of the P system, which has much retinal redundancy, is less susceptible to elevated tension than the larger M ganglion cells.

There have been a number of good results obtained with achromatic stimuli but these have not been linked with the performance of the M_V pathway and it is now of some interest to see if the stimuli, found to be most effective in these tests, are those that are optimum for M_V cells. In agreement with the present results one study (Ross *et al.*, 1984) showed a significant threshold elevation when glaucomatous subjects (with scotoma and disc cupping) viewed a 10 Hz and 0.5 c deg⁻¹ grating. In a similar series of tests (Atkin *et al.*, 1979), threshold elevation was observed in 50% of ocular hypertensives tested at 8 Hz and 1.2 c deg⁻¹. Another study (Tyler, 1981) showed that subjects with elevated intra-ocular tension displayed significant threshold elevation for seeing a 2° spot, that was modulated at frequencies around 30 Hz, and viewed at a retinal eccentricity of about 15°, in the region where Bjerrum's scotoma forms. The proportion of elevated thresholds diminished from 95 to 60% when the test spot was moved from 15° eccentricity to the central retina. All of these experiments had similarities to the present series although, for most stimulus conditions, the frequency-double effect was either weak (Atkin *et al.*, 1979; Ross *et al.*, 1984) or not visible (Tyler, 1981). Now, in hindsight, it is apparent that good results can be obtained when the visual stimuli approached the optimum spatiotemporal conditions for the frequency-double percept.

The psychophysical results from human subjects are mimicked by results obtained from macaques with artificially-induced glaucoma, where visual performance is estimated from the form of the pattern electroretinogram (PERG) or the pattern visual evoked potential (PVEP). One study (Marx *et al.*, 1988), using a grating modulated at 6 Hz, found that the signal from the affected eye suffered greatest reduction at 0.5 c deg⁻¹ the lowest presented spatial frequency. A second study (Johnson *et al.*, 1989),

with artificially-induced glaucoma, presented checker boards at a contrast reversal frequency of 10 Hz and found that the greatest response depression occurred for 2° wide checks. With checker boards the fundamental spatial frequency component occurs along the diagonals of the check pattern or, for a 2° check, at $1/\sqrt{8} = 0.35$ c deg⁻¹, which once again is close to optimum for the production of the frequency-doubled illusion in the peripheral retina. Johnson *et al.* (1989) also found a very strong correlation between the PERG responses and the number of fibres remaining in the optic nerve of the affected eye, but this relationship may only hold for instances of severe damage and the test may not be sensitive enough for the early detection of glaucoma (H. A. Quigley, personal communication) due to technical difficulties associated with PERGs.

Whether the percept of the frequency-doubled illusion is actually necessary to achieve the best results requires some further investigation. For example as subjects adjust the contrast upward from 0 they usually report the perception of an amorphous flicker before being able to see the frequency-doubled percept. Experiments in which the limen was more controlled, that is to say where different thresholds were assessed, say 40, 60, 80% correct, might reveal some particular percept along the route from low to high contrast to be best suited to glaucoma detection. At least some indication of which threshold is best can be obtained by examining the thresholds from the two methods used here. The thresholds for the two highest modulation frequencies of method 1 (Methods) were lower than those obtained for the method of adjustment, while the thresholds for method 1 for lower frequencies were higher than those obtained at the same frequency by the method of adjustment (method 2). Given that overall the thresholds obtained from method 1 were less significant determinants of glaucomatous damage than those of method 2 might suggest that examining slightly different measures of threshold may not be very fruitful. For example, we have also examined some super-threshold performance measures (unpublished) and these did not appear to perform as well as method 2.

The results of the present study would seem to suggest that good results from past experiments in the early detection of glaucoma can usually be related to the production of the frequency-doubled illusion or to the activation of the M_V pathway. Our results support this

belief and there are now compelling reasons for examining the M_V pathway for early signs of glaucoma. These include the sparseness of the retinal distribution of M_V ganglion cells, their susceptibility to elevated intraocular tension, the capacity to selectively test their performance through the frequency-doubled illusion, the probability that other clinical conditions selectively affect the P rather than the M pathway and, finally, the ability to test M_V cell performance over an extensive area of retina with the use of low spatial frequencies means that the test is not dependent on the observer being particularly well refracted. All of these factors when taken with the small individual variance found in the present study would indicate there is the potential to develop a practical clinical test using the frequency-doubled illusion.

Acknowledgement—TM was supported in part by a Canadian Medical Research Council Fellowship.

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