The pattern electroretinogram in glaucoma and ocular hypertension

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Abstract
Thirty one eyes with established glaucoma, 61 high risk ocular hypertensive (OHTs) eyes, 66 medium risk OHT eyes, 58 low risk OHT eyes, and 47 control eyes have been followed for up to 2 years by clinical examination and pattern electroretinography (PERG). The study was 'masked' so electrophysiological and clinical data were kept separate. Criteria have been devised which enable PERG measurements to distinguish all established glaucomatous eyes from all normal controls; these criteria demonstrate abnormalities in some OHT eyes, particularly those at high risk. The PERG abnormality is greatest in eyes with established glaucoma in which the intraocular pressure has been lowered by treatment. The PERG becomes smaller as the degree of clinical abnormality increases. Test-retest variability of the PERG is sufficiently low to ensure that most of those first described as abnormal continue to be so.

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The standard definition of glaucoma (raised intraocular pressure (IOP) changes in the disc and a characteristic field defect) cannot be made until a considerable proportion of the ganglion cells in the retina are dead. However, it is known that by the time the diagnosis is made, the underlying condition of ocular hypertension (OHT) may have been present for up to 10 years so the diagnosis records an arbitrary point (determined by the sensitivity of the diagnostic methods) in a continuing decline. There have been two recent reports which suggest that treatment of glaucoma suspects, begun earlier, reduces IOP and in follow-up, the rate of development of scotomata decreases, compared with untreated OHT. It is therefore possible that by treating OHT itself, and not waiting for diagnosis by perimetric means, the appearance of scotomata could be largely prevented. The problem with this suggestion is that there are a very large number of patients with OHT, and only a small proportion will develop field defects in the near future. Universal treatment is therefore most undesirable. What is required is a means of detection of lesser degrees of functional defect than is possible with perimeters. Some tests proposed to fill this gap (measurement of defects in the nerve fibre layer for example) require considerable skill to record and to interpret. They record structural retinal or optic nerve damage. It would be desirable to develop functional tests to measure very small changes from normal.

First reports have suggested that this requirement is fulfilled by an objective electrophysiological test of visual function, the pattern electroretinogram (PERG).a,b We and others have shown that the PERG is abnormal in patients with established glaucoma. In addition, colour vision tests appear to discriminate between normals and patients with glaucoma. What is required is a prolonged and careful investigation into the progress of the disease as monitored by these tests in a larger cohort. The aim to establish whether the tests can be used in the management of patients with various grades of OHT. We report below on results with the PERG (another paper describes results obtained by testing macular colour vision).c

Methods

RECRUITMENT
We aimed to recruit approximately equal numbers of patients with established glaucoma, high risk, medium risk, and low risk glaucoma suspects, and normal subjects. The glaucoma patients were recruited from the glaucoma clinic: all eyes had typical reproducible glaucomatous field defects. The glaucoma suspects were classified as high, medium, or low risk according to the level of their IOP and cup:disc ratio (CC:D) (see Table 1 for exact criteria). Yablonski has shown that these groups have different rates of conversion to glaucoma and that the higher the IOP and the C:D ratio the greater is the risk of developing glaucoma. Recruitment of patients with established glaucoma and glaucoma suspects was performed by a clinical fellow who was not concerned with the PERG testing. The normal subjects were recruited from patients'
spouses, hospital staff, and subjects referred to other hospital departments with minor uniconular complaints and essentially normal examination results. Thus, one eye was chosen from each normal subject for testing.

EXCLUSIONS
Patients were excluded if they suffered from other eye disease or severe systemic disease. Patients’ eyes with visual acuity <6/9 were excluded: these failures were due to cataract, amblyopia, or other uniconular conditions. Four individuals who failed to attend the follow-up were also excluded. By December 1990, 31 glaucoma eyes from 21 subjects, 61 high risk eyes from 41 subjects, 66 medium risk eyes from 43 subjects, 58 low risk eyes from 33 subjects, and 47 normal eyes from 47 subjects had been recruited to this study and examined as described below over a minimum period of 1 year. Sixty eyes from 138 patients were excluded on the grounds indicated above.

CLINICAL TESTS
All patients were examined every 6 months. At each visit visual acuity, application tonometry, and full clinical examination of fundus and disc were carried out. Perimetry was carried out with the Humphrey perimeter, program 24/2, and analysed statistically. In all cases disc photographs were taken annually both in full colour and in red-free light. A fasting blood glucose test was performed on the first visit to identify any subject with diabetes mellitus. If this diagnosis was subsequently confirmed, the patient was excluded from the special tests.

SPECIAL TESTS
Patients were referred to the electrodagnostic department for PERG testing. No clinical information was sent with the patients. The technique for recording PERGs has been described previously. 8,17,22 The PERG was recorded bilaterally, using gold foil corneal electrodes and a reference electrode on the skin near the lateral canthus. The stimulus subtended 16°×22° and the pattern reversing checks were of 30° subtense and near 100% contrast. There were six reversals per second. About 250 sweeps were averaged for a response; four or more responses were recorded. The initial cornea positive response (PS0) and the later corneal negative response (N95) were measured separately (see Fig 1). In addition, in many of the patients’ PERGs were recorded with reversals every 60 ms to obtain a ‘steady state’ response, which approximates to a sine wave.21,27 The time base was lengthened to 180 ms, so three consecutive cycles of response could be visualised during the averaging. Records were obtained with the whole screen exposed (16°×22°) and also with an 11° opaque disc placed at the screen centre to remove the stimulus from the central 27% of the retina previously stimulated. Colour contrast sensitivity tests were carried out as described in the accompanying paper.27

Tests were carried out by technicians who measured the results. This was cross checked by one of us, who also made the (rare) decision that some results had to be discarded. This occurred if there was inadequate documentation, or internal evidence of inconsistency and poor recording: the most common causes of the latter are mains artefacts and epiphor. Electrodiagnostic reports were entered into the patients’ files but were not used in management. Results were entered into a database by the technicians, and again these were rechecked, and a number of anomalous results found to be due to entry errors. At a different time, clinical results were entered under the supervision of the clinical fellow and entry errors removed by rechecking. Although the clinical fellow was aware of the relationship between all results, apart from this the study was masked and decisions about treatment of patients’ eyes were made by persons other than those involved in the tests.

In an attempt to quantify the reproducibility

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**Fig. 1** Diagram of normal transient PERGs illustrating how measurements of negative and positive waves were made.

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<table>
<thead>
<tr>
<th>Table 2A</th>
<th>Mean values of four PERG parameters for the right eyes of glaucoma patients, high, medium, and low risk suspects. Also mean values of PERG parameters in 47 normal eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class</strong></td>
<td><strong>Eyes (n)</strong></td>
</tr>
<tr>
<td>Glaucoma</td>
<td>15</td>
</tr>
<tr>
<td>SD 15</td>
<td>0.66</td>
</tr>
<tr>
<td>High risk</td>
<td>30</td>
</tr>
<tr>
<td>SD 10</td>
<td>0.80</td>
</tr>
<tr>
<td>Medium risk</td>
<td>31</td>
</tr>
<tr>
<td>SD 10</td>
<td>0.79</td>
</tr>
<tr>
<td>Low risk</td>
<td>30</td>
</tr>
<tr>
<td>SD 11</td>
<td>0.89</td>
</tr>
<tr>
<td>Normals</td>
<td>47</td>
</tr>
<tr>
<td>SD 11</td>
<td>0.77</td>
</tr>
</tbody>
</table>

NB The ratios N/P are the means of the individual values; they are not therefore identical to the ratios of the means shown in the columns to the left. Also note that only one eye of each normal subject was tested; therefore the normal values are identical in Tables 2A and 2B.

<table>
<thead>
<tr>
<th>Table 2B</th>
<th>Mean values of PERG parameters for the left eyes of glaucoma patients, high, medium, and low risk suspects. Also mean values of PERG parameters in 47 normal eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class</strong></td>
<td><strong>Eyes (n)</strong></td>
</tr>
<tr>
<td>Glaucoma</td>
<td>16</td>
</tr>
<tr>
<td>SD 14</td>
<td>0.47</td>
</tr>
<tr>
<td>High risk</td>
<td>31</td>
</tr>
<tr>
<td>SD 10</td>
<td>0.91</td>
</tr>
<tr>
<td>Medium risk</td>
<td>35</td>
</tr>
<tr>
<td>SD 12</td>
<td>0.80</td>
</tr>
<tr>
<td>Low risk</td>
<td>28</td>
</tr>
<tr>
<td>SD 11</td>
<td>0.76</td>
</tr>
<tr>
<td>Normals</td>
<td>47</td>
</tr>
<tr>
<td>SD 11</td>
<td>0.77</td>
</tr>
</tbody>
</table>

NB The ratios N/P are the means of the individual values; they are not therefore identical to the ratios of the means shown in the columns to the left.
of the PERG, in 40 eyes additional PERG recordings were made within 4 weeks of the first. The eyes thus tested were drawn in equal numbers from all the groups described above. The conditions for testing the time of day and the technique were the same at each visit. The technicians conducted the test at random and were unaware of the previous results.

Results

The cohort investigated and the results of the first test are shown in Tables 2A and 2B. The mean age of the established glaucoma patients and glaucoma suspects was 55 years: the mean age of the normals was 41. The small difference in the ages will make no significant change to the results. It has been shown that below the age of 70, the PERG amplitude changes little with age.27 28 The PERG is a biphasic response with a cornea positive wave peaking at around 50 ms after the stimulus and a cornea negative wave at about 95 ms. We have derived four parameters from the recording, that is, P50 (μV), N95, (μV), the total PERG amplitude (N+P), and the ratio N/P. For eyes with established glaucoma the mean values of all these parameters are smaller than for normals. In each of the glaucoma suspect groups, the mean P50, N95 and (N+P) are smaller than for normal subjects. The difference between the normal eye values and the values of the eyes in the suspect groups is greatest for the high risk and least for the low risk group. The mean results and standard deviations for cohort using all the parameters of the PERG are shown in Tables 2A and 2B. Table 2A shows the results for right eyes only and Table 2B shows those for left eyes only. A statistical analysis was carried out on the results obtained from the right eyes of all patients in the cohort (Table 3). Table 3 shows the results for each parameter of the PERG tested. In this table t tests have been done between each group and the starred values are highly significant. Eyes with established glaucoma have PERG parameters which are significantly different from all the other groups of eyes tested. The ‘t’ values are very high. For the high risk suspect eyes, all parameters of the measured PERG differ highly significantly from normals and the highest t value corresponding to the most significant difference is for N/P. For medium risk eyes, both P50 and (N+P) are significantly different from normal values. The t value for (N+P) in low risk eyes is different from normal eyes at the 5% level of significance.29 An analysis corresponding to that shown in Table 3 was also performed for the data on left eyes, with similar results. Left eye results are not given in Table 3 because of this. The differences between the grouped data for right and left eyes were small, providing the data related to the risk class. The largest differences between the mean values for right and left eyes were seen in the high risk suspect groups, but they were not significant using t tests. (For P50 t=0·88 and for (N+P) t=1·55, df=62).

Thus, in grouped data, the amplitude of the PERG reflects the progress of the condition. It should be noted that for the established glaucoma eyes the mean IOP is 17·4 (SD 3·76) mm Hg, lower than in the low risk OHT group (18·7 (SD 5·7)). This would be expected since all glaucoma patients were treated. In the high and medium risk OHT patients, some are treated, but in most cases pressure is considerably raised. The mean level is 24·0 (SD 4·8) mm Hg. The results therefore indicate a correlation between glaucomatous damage and the PERG amplitude, rather than a correlation between increase in pressure and the PERG, or some other interfering variable such as poor vision hindering cooperation in the glaucoma patients.

In persons with asymmetrical glaucoma, the PERG parameters differ in amplitude but within a risk class the results from the right and left eyes of this cohort were very similar, which suggests that the results from the two eyes are largely independent and related to the severity of the disease. The analysis shows that the reduction in PERG parameters progresses with risk factors.

In order to assess which of the PERG parameters are most effective at detecting glaucomatous damage, scatter diagrams plotting N95 versus P50 (N+P) versus N/P and (N+P) versus N/N+P for 47 normal eyes and 31 glaucoma eyes were constructed (Figs 2, 3, and 4 respectively). Figure 2 shows that P50 alone is quite a good discriminator for glaucoma although there is some overlap between normal and glaucomatous eyes. Better discrimination between normal eyes and glaucomatous eyes is seen in Figure 3. The parameter (N+P) allows better separation of the two populations than P50 alone. If a value of (N+P) of 2·7 μV is chosen 29 of 31 glaucoma eyes have (N+P) values below this and all the normal eyes have (N+P) values above. The ratio of N/P is not such a good discriminator, although there is a definite trend for glaucomatous eyes to have smaller N/P values than normal eyes. A more mathematically correct ratio (N/N+P) was chosen and plotted against (N+P) in Figure 4. However this ratio was no better a discriminator than N/P. In summary, in this cohort, the parameter (N+P) allows the best

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**Table 3**

<table>
<thead>
<tr>
<th>Category</th>
<th>Low risk</th>
<th>Medium risk</th>
<th>High risk</th>
<th>Glaucoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
<td>N95</td>
</tr>
<tr>
<td>N/P</td>
<td>1-00</td>
<td>1-04</td>
<td>3-07*</td>
<td>0-88</td>
</tr>
<tr>
<td>Medium</td>
<td>0-54</td>
<td>0-54</td>
<td>0-54</td>
<td>0-54</td>
</tr>
<tr>
<td>High</td>
<td>0-61</td>
<td>0-60</td>
<td>0-60</td>
<td>0-60</td>
</tr>
</tbody>
</table>

*Indicates that a test between the mean value for this group and the one in the left-hand column shows a difference with a probability p<0·02.
Discrimination between glaucomatous and normal eyes.

Table 4 shows the mean PERG parameters for each group of eyes for the first and second visits.

The second test occurred 6 months after the first and these second test results might be expected to give an indication of progression over time. It can be seen that the differences between the groups of eyes which were apparent on the first test are reproduced on the second test. In the high risk suspect eyes and established glaucomatous eyes, there is an overall decrease in the mean amplitude of the PERG. It can be seen that there are very small differences between the successive visits. For the medium and low risk groups the largest proportional decline is in the N95 measurement, indicating this may be preferentially affected in the early stages of OHT and glaucoma.

RETAIL AREA TESTED
Following Watanabe et al., we recently investigated the central and paracentral components of the PERG in our cohort, using 'steady state' PERGs. Sixty six persons gave useful results on 130 eyes. These results were obtained between August and December 1990. Table 5 shows that the differences described between risk classes for the transient PERG are reproduced for the steady state ERG. The ratios between steady state and transient voltages are not significantly different for any class. The contribution of the central 11° of the retina is about equal to that of the remaining 75% of the screen, for each risk class. The apparent relative increase of the surround response in the glaucoma patients is not significant. Watanabe et al. who dealt with a smaller sample, and compared controls to eyes with established glaucoma, obtained a different result: the response from the peripheral retina decreases more with disease. Our own work, with luminance and colour contrast sensitivity, suggested to us that the peripheral retinal PERG would be more affected in the glaucoma patients than others: however, this expectation has not been fulfilled. A possible explanation is that the amplitude of the PERG is so small in glaucoma, especially when the macula and perimacular regions are not stimulated, that the contamination of the responses by noise becomes more important.

INDIVIDUAL RESULTS
It can be seen in Tables 2 and 5 that the standard deviations of the PERGs are about 25% of the mean values. It was decided to assess carefully the reproducibility of the PERG. Test-retest investigation of the PERG was carried out by repeating tests on 40 patients' eyes within 4 weeks of the first test. The eyes thus tested consisted of equal numbers from each of the three groups and from normal and glaucoma groups. On the first occasion, 16 eyes had abnormal PERGs, and 24 normal responses. The definition of abnormality was (N+P)<2.7, a criterion derived from analysis of all the PERGs recorded, which is further explained in the next section. The mean (N+P) for the first test was
The values compared to the second sets of values. The regression coefficient=0-80. The formal calculation of the reliability coefficient (1-square of SD of differences)/square of SD of first measurement)=0-82. Thus test-retest variability should not cause significant differences in the results.

The correlation between clinical estimates of normality and estimates derived from the PERG depends upon the PERG criteria used to divide eyes into normal and abnormal groups.

To attack this problem, we sorted out results, using the facilities of the spreadsheet, using various criterion levels to separate 'normal' from 'abnormal'. Results for the entire cohort are shown in Figures 6, 7, 8 and 9. It can be seen that for eyes of patients with established glaucoma, the total peak-to-peak amplitude of the PERG is nearly always lower than for all the normals; for the high risk suspect eyes, the overlap is considerable, and for low risk it is nearly complete. By adopting various criteria for normality, one can alter the sensitivity and specificity of the test, as shown in Table 6. If a criterion of P50<1.8 μV is chosen, 2% of normal eyes will be considered abnormal, but only 69% of eyes with established glaucoma will be detected. More convincingly, if abnormality is defined as N90<0-6 μV, then 2% of false positives will be detected, and 84% of all glaucomatous eyes will be abnormal.

Better criteria can be obtained if several measurements are made on the PERG. Our data show that for one determination, abnormality is best defined as (P+N)<2-7 μV, and N/P<0-7. Under such circumstances, no eye from the control group is considered as abnormal, and 94% of eyes of patients with established glaucoma are detected.

The same result can be applied to the data from ocular hypertensives, and there it can be seen that a much lower proportion are considered abnormal, and more than twice as many eyes at severe risk have abnormal PERGs as those in lower risk groups.

SUBSEQUENT VISITS
Glaucoma suspects have repeated field tests, and only when a possible defect is subsequently confirmed is it considered that the patient has changed from OHT to established glaucoma. In just the same way, we can combine the results of two or more PERG tests. If abnormality is defined by the PERG falling below (P+N)<2-7 and N/P<0-7 on one occasion during the entire investigation, the results are as shown in Figure 10: the main difference is that now, 100% of normal eyes can be distinguished from 100% of the eyes of patients with established glaucoma.

Discussion
The present results extend and confirm our (and other) prior reports of PERG abnormalities in glaucoma and ocular hypertension. In particular, we have carried out a masked study in a relatively sizeable cohort for up to 2 years, and have been able to observe and correlate changes in the PERG with the clinical condition. The number
of suspects and normals tested is larger than in our previous report and the follow-up time is at least 1 year in all cases. In addition, while in previous work patients' results have been compared with previous established normal values, we have attempted to establish which criteria would be of greatest clinical value in our cohort. We have demonstrated that the PERG is persistently abnormal in eyes with established glaucoma. In our cohort, using as a criterion of abnormality (P+N)<2.7 and N/P<0.7, we were able to separate 94% of such eyes from the normal group with one PERG test. With two tests we were able to differentiate 100% of the glaucomatous eyes from normals. Although this sensitivity and specificity is striking and compares well with automated perimetry, it must be treated with some caution. Our sample size is relatively small and our recruitment criteria removed patients with early cataract or macular degeneration who would normally be included in a glaucoma clinic. Nevertheless the result is objective in a sense that a field test is not. It is as easy for the patient and at least in our cohort seems as reliable as perimetry. Therefore it is likely that serial PERGs can have a place in management of glaucoma patients and suspects.

Our results also demonstrate that glaucoma suspects' eyes show a reduction in their PERG recordings. All our glaucoma suspect groups had smaller mean PERGs than the normals, although this was significant for the high risk group only. The reduction in the PERG of suspects eyes was notable because of the proportionately greater reduction in the N95 component. This had been noted in our previous report and suggests the N95 wave is selectively damaged by the glaucomatous process.

The trial was conducted so that clinical data and the PERG results were collected independently. However the reduction in the PERG mirrored the degree of risk assessed clinically. Thus, high risk suspect eyes had three times as many abnormal PERGs as the low risk eyes; the PERG abnormalities were reproduced on testing at the second visit. This suggests that abnormal PERGs truly reflect abnormal retinal function in glaucoma suspects' eyes. This abnormality was detected while field tests, which are currently used to monitor function, were completely normal. The reasons for the sensitivity of the PERG to retinal or optic nerve damage are not fully understood. Several factors must contribute: the amplitude of the response is due to sampling from a large retinal area, so point-to-point variation in sensitivity is averaged out. Furthermore there is some evidence that the PERG is produced by larger diameter retinal ganglion cells which are known to be affected earlier than small ganglion cells in the evolution of glaucoma. The steady state or peripheral retinal responses in our hands are no more effective criteria, though our experience of them is not so extensive. These results are of interest insofar as they may give some clues about the underlying pathology. For example, the P50 response is affected in glaucoma although it is generated in part by the interneurons or glia of the retina, and only partly by ganglion cells: this suggests that increased pressure may do more than damage optic axons near the cribiform plate.

Although the PERG is an objective test, the voltage is small and the recordings contaminated by noise and artefacts. We have used various methods of stimulation and recording in order to identify which is the most efficient method. However, no one method seems to have any obvious superiority. Our results with test-retest...
show acceptable reproducibility: although there is some variability in each individual's results on repeat testing, this is only very infrequently of a degree which would cause a previously normal result to be subsequently classified as abnormal, or vice versa. We have reported the results of PERG tests on a sizeable cohort followed for 1 year. The follow-up of these patients is continuing; this will provide further information on the natural evolution of glaucomatous disease as monitored by the PERG. It will allow us to correlate the PERG results with the subsequent development of perimetric deficits and enable us to determine whether treatment of glaucoma suspects with topical β blockers has an effect on the PERG.

In summary, the PERG is as quick to perform as a test of the visual field with automated perimetry. It is subject to some variability, but this is of an acceptable degree. The PERG convincingly separates all persons with field defects from normals and also demonstrates that a proportion of suspects have reduced responses. The proportion of abnormal PERG mirrors the degree of risk of the suspects. Our experience suggests that an abnormal PERG is an indicator of retinal damage during the phase of 'preglaucoma' before any scotoma can be detected by perimetry. Logic suggests that the PERG could be used for screening in this population, but to demonstrate its value in such a role would require a larger and more prolonged controlled clinical trial.

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