ORIGINAL ARTICLES—Laboratory science

Functional imaging of the retina using the multifocal electroretinograph: a control study

S Parks, D Keating, T H Williamson, A L Evans, A T Elliott, J L Jay

Abstract

***Background*—A new technique exists that enables functional mapping of the retina. A control population was examined to obtain normative values and to assess the reproducibility of this new test.**

***Methods*—Twenty healthy volunteers were tested using a 61 hexagonal array stimulus with a 14 minute recording period. Median 5th and 95th percentiles were determined for implicit times and amplitude measures for the 61 test areas. Repeat measurements were performed on 10 individuals. Wilcoxon and Bland and Altman techniques were used to quantify reproducibility of the test.**

***Results*—The implicit time of the waveform components was not found to vary over the retina (peak or b-wave component, 35.52 (1.4) ms; trough or a-wave component, 17.76 (0.8) ms). Reproducibility was found to decrease with eccentricity (coefficient of repeatability 17.4% for the central area increasing to 30.3% for the peripheral ring).**

***Conclusions*—The findings suggest that reproducibility, although variable with eccentricity, is comparable with conventional electrophysiology. These limits of variation were used to assign confidence intervals to individual retinal areas and will be used (future work) in the examination of diseased states.**

(*Br J Ophthalmol* 1996;**80**:831–834)

Tennent Institute of Ophthalmology, West Glasgow University NHS Trust, Glasgow
S Parks
D Keating
T H Williamson
J L Jay

Department of Clinical Physics and Bio-engineering, University of Glasgow
S Parks
D Keating
A L Evans
A T Elliott

Correspondence to:
S Parks, Tennent Institute of Ophthalmology, Western Infirmary, 38 Church Street, Glasgow G11 6NT.

Accepted for publication
24 May 1996

The conventional electroretinogram (ERG) is usually elicited from a flash stimulus and records hyperpolarising and depolarising activity from the retina. The waveform possesses several distinct components such as the a-wave, a negative signal generated by the photoreceptors and the b-wave, a positive signal generated by the Müller cells. Although the ERG is used routinely in the diagnosis and monitoring of a wide range of retinal disorders its application is restricted because the diffuse stimulation of the retina evokes a global response, thereby preventing the detection of localised abnormalities. The focal electroretinogram (FERG) has been applied to try to overcome this limitation of the ERG. The FERG is evoked by a small area of the retina (10 degrees or less)[1] and most commonly involves stimulation of the retina by a small flickering light with a steady background illumination contained within a modified ophthalmoscope.[2] Unfortunately this method suffers from prolonged recording times and varying signal to noise ratios.

A new technique has been described which overcomes some of the shortfalls of FERG. The visual evoked response imaging system (VERIS)[3] allows functional mapping of the retina by the ERG. The method enables simultaneous recording from a large number of retinal areas. Each area is independently stimulated in a sequence employing pseudo random binary stimulation (PRBS). The sequences of stimulation are uncorrelated (achieved by temporal modulation of the sequence for each area); therefore, the individual responses from different areas of the retina can be extracted (Fig 1). The amplitudes of the waveforms of these signals are used to produce functional three dimensional plots of the ERG responses of the retina (Fig 2).

The aim of this study was to establish normal ranges for the multifocal ERG from a control population for 61 areas of the retina within a 25 degree visual field and to examine the reproducibility of the results obtained.

Methods

Twenty normal healthy volunteers were examined using the multifocal ERG. Ten of the subjects were examined on two different occasions. The stimulus array was presented on a multiscan monitor (75 Hz) positioned 32 cm

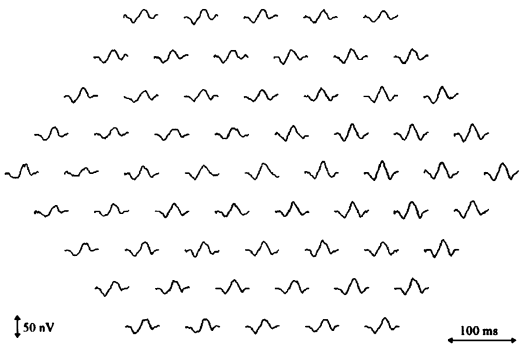

Figure 1 Trace array of 61 local responses from multifocal electroretinogram.

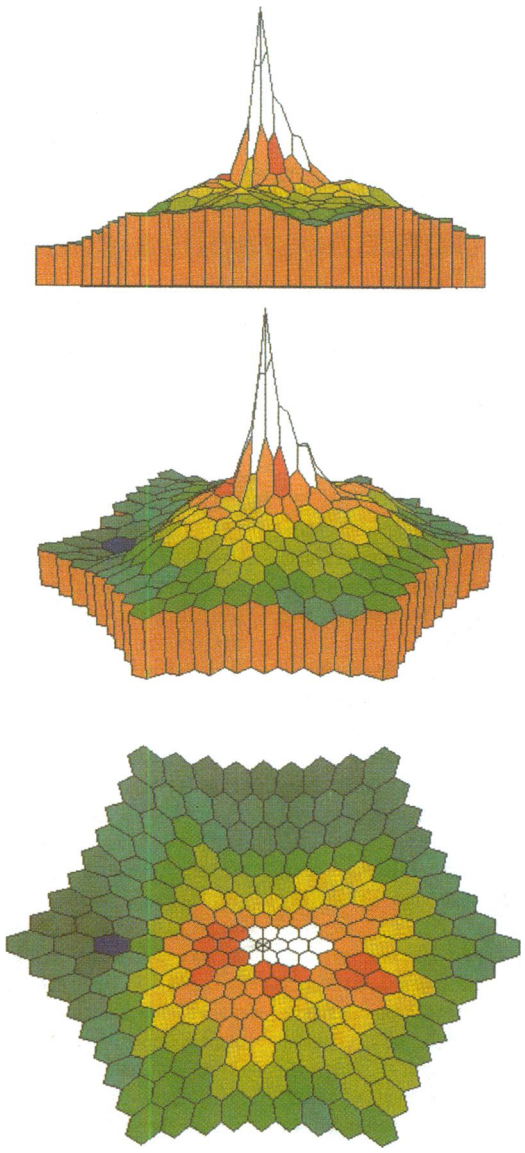

Figure 2 'Three dimensional' scalar product plot obtained from the left eye of normal subject showing a 'hill of vision' representing increased responses centrally.

from the subject's eye. The array contained 61 hexagons (scaled for eccentricity) which covered an area of a 25 degree visual field (Fig 3). A steady background luminance filled the periphery of the display and a central cross was used to maintain fixation. Each hexagon alternated between black and white (using 89% contrast) in a binary m-sequence. The luminance ERG employed a gain of 160 000 with an ADC digitisation rate of 675 Hz and a high/low pass filter of 1–300 Hz. Recordings were made with monopolar H-K loop scleral electrodes. These electrodes match the stability of skin electrodes, are as sensitive as gold foil electrodes[4] and, in our experience, cause less discomfort than contact lens electrodes. A ground electrode was placed on the forehead of the subject and reference electrodes placed on both outer canthi. The recording period comprised 20 intervals of 43 seconds providing a total recording time of 14 minutes 20 seconds.

The local ERG response of the retina was extracted for 61 stimulated areas using a fast m-transform algorithm.[3] The median, 5th, and 95th percentiles of the peak and trough waveform latencies and amplitudes, and scalar product of the waveform for the 20 controls were calculated for each of the 61 areas. Non-parametric statistics were employed because data in conventional electrophysiology have demonstrated non-normal distributions.[5]

Amplitudes and latencies were determined using a specially designed computer program. The amplitude measure (scalar product method)[3] is calculated from the entire local response waveform and is therefore less susceptible to noise than simple peak to peak amplitude measurements from only two points. The VERIS system allows the estimation of an amplitude measure which is the scalar product (a simple multiplication and summation) of each local response with the subjects' normalised global response. However, by creating a normalised response template from the control data, and so creating an ideal waveform, an amplitude estimate was produced that was less susceptible to noise and a more accurate estimate of deviation from normative values. The Wilcoxon matched pairs test was used to examine the results of scalar product values obtained using the median template in the reproducibility study. Further analysis of the repeated measurements based on the definition of a repeatability coefficient adopted by the British Standards Institution[6] was performed. The coefficient of repeatability was defined as the standard deviation of the mean differences between pairs of repeated measurements divided by the average of the means of the two tests. All subjects were recruited from the Tennent Institute of Ophthalmology and informed consent and ethics committee approval obtained.

Table 1 Minimum and maximum median values for scalar product, peak and trough waveform latencies, and amplitudes, and their respective confidence levels

	Area No	*Median*	*5th percentile*	*95th percentile*
Min trough latency (ms)	18 areas	16.28	13.32	20.72
Max trough latency (ms)	5, 27, **28**, 36	19.24	16.13	22.42
Min trough amplitude (nV)	**28** (blind spot)	11	4.75	20.1
Max trough amplitude (nV)	35	22	9.95	36.2
Min peak latency (ms)	34, 42	29.6	27.53	34.18
Max peak latency (ms)	1, 6, 27, 36	35.52	29.23	38.48
Min peak amplitude (nV)	**28** (blind spot)	27	10.7	45.6
Max peak amplitude (nV)	61	47.5	25	62.9
Min scalar product (nV/deg^2)	**28** (blind spot)	4.95	1.5	11.80
Max scalar product (nV/deg^2)	**31** (central area)	55.84	27.5	82.27

Results

The mean age of the volunteers was 31.5, range 16 to 52 years with nine males and 11 females. The medians of the peak and trough waveform latencies and amplitudes, and scalar products from the 61 areas of the left eye are shown in the appendix. The minimum and maximum median values were calculated and their respective percentage confidence levels are shown in Table 1.

The largest median value for the waveform trough latency and the minimum median values for the peak and trough waveform amplitudes were, as expected, within area 28 (an area the majority of which covers the blind

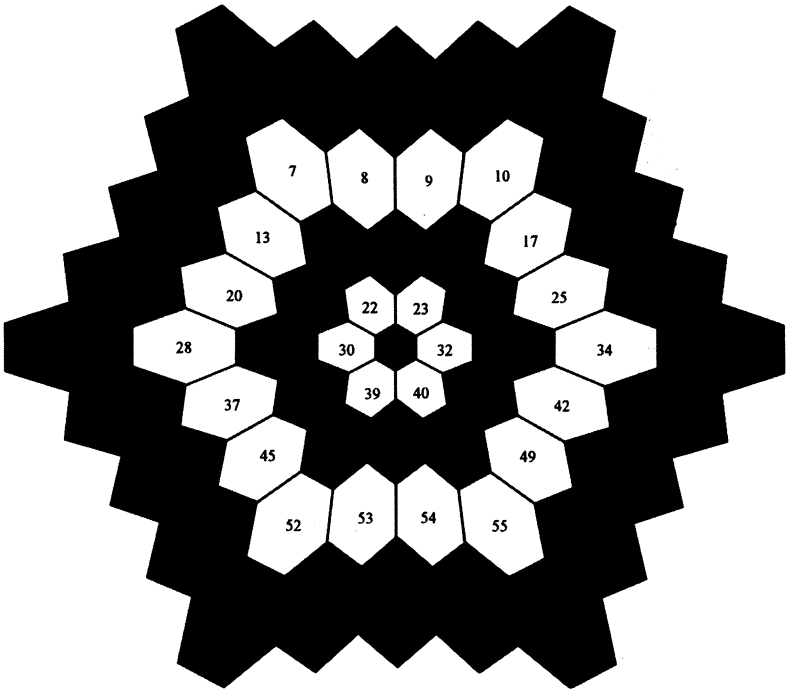

Figure 3 The stimulus array of 61 hexagonal areas with concentric rings indicated by alternate shadings.

spot). The spread of normal ranges was comparable with previous studies employing the conventional ERG.[5] In the reproducibility test significant differences in the results were only found in two of the 61 areas (areas 18 and 24), a result which might be attributed to chance. In order to simplify data analysis the data were split into a set of four concentric rings and the remaining central area. The coefficients of repeatability for each ring are quoted in Table 2.

Table 2 Coefficients of repeatability for concentric rings

Coefficient of repeatability (%)	*Degree of eccentricity*	*Area Nos*
17.8	Central area	31
23.4	First ring	22, 23, 32, 40, 39, 30
22.2	Second ring	14, 15, 16, 24, 33, 41, 48, 47, 46, 38, 29, 21
26.8	Third ring	7, 8, 9, 10, 17, 25, 34, 42, 49, 55, 54, 53, 52, 45, 37, 28,20,13
30.5	Outer ring	1, 2, 3, 4, 5, 11, 18, 26, 35, 43, 50, 56, 61, 60, 59, 58, 57, 51, 44, 36, 27, 19, 12, 6

Discussion

In this control population the implicit time of the peak and trough waveform components (peak 35.52 (SD 1.4) ms, trough 17.76 (0.8) ms) did not vary significantly over the retina (the ADC sampling rate used was 675 Hz giving discrete intervals of 1.48 ms which is greater than the largest standard deviation of 1.4 ms). Differences in both peak and trough waveform amplitude were found but this is not surprising as the stimulus array is designed only to approximate the distribution of receptors in the retina with increasing stimulus area with eccentricity. The ranges given for amplitude and latency measurements compare favourably with conventional electroretinography.[5]

The Wilcoxon test showed good agreement in the data distributions for repeat measurements with only two of the 61 areas tested showing significant differences. Actual values for the limits of repeatability were calculated using the technique described by Bland and Altman.[7] The results show these limits increase with eccentricity. The reason for this finding remains obscure but may be related to the different distributions of cones within each hexagonal area. The findings suggest that only large differences in serial results will be detectable with the current system and that care must be applied when subtle differences in the results are detected, for example, in disease groups. Further improvements in repeatability could be expected by grouping areas together or by improving the signal to noise ratio. In this study retinal illumination was not controlled nor pupil diameter monitored during the measurement for three main reasons. Firstly, as the aim of the study was to obtain normative values for a routine clinical test, patient comfort and ease of acquisition were paramount. The inclusion of dilatation and refraction would have resulted in a more complex protocol of at least twice the duration. Secondly, photoreceptor density between individuals is highly variable (recent evidence suggests cone density can vary by as much as 30%).[8] While it is not under dispute that signal amplitude is dependent on retinal illumination it does not necessarily follow that equalising retinal illumination within individuals will lead to responses of equal amplitudes. Finally, the authors wished to exclude the possible artefactual influence of external correcting lenses (unpublished data) which might disturb the pattern of stimulation of the retina.

The scalar product method was used to assign confidence intervals to individual retinal areas and will be used in the examination of disease states to produce functional maps with significant defects clearly defined. For example, if a result falls outside the 95% confidence limits this can be represented by a different colour on the map of ERG function.

The test provides and should allow the extent and severity of disorders which produce abnormalities in the ERG to be investigated. In this study only the photopic luminance ERG was used, thereby primarily examining the retinal cones. By changing the stimulus array and recording factors of the technique, retinal maps could be generated from the rod system or from the ganglion cells. In this way the technique offers the possibility of layer by layer functional mapping of the visual pathway in the retina. The system has potential in the clinical setting because relatively short protocols of only 15 minutes are required and produce recordings from both eyes simultaneously. With the use of H-K loop scleral electrodes signals can be produced with minimal patient discomfort.

In conclusion, the VERIS system provided individual ERG waveforms from 61 areas of a 25 degree visual field. The reproducibility of the results suggests moderate variation be-

tween tests, therefore further development of the technique will be required. Even so the method shows considerable promise for further investigation of retinal diseases.

This work was supported by Scottish Home and Health Department grant K/MRS/50/C2336.

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Appendix

		Latency (ms)		*Amplitude (nV)*		
Area No	*Scalar product (nV/deg²)*	*Trough*	*Peak*	*Trough*	*Peak*	
1	6.34	17.76	35.52	13	35.5	
2	6.71	17.76	32.56	14	30	
3	7.17	16.28	32.56	13	30.5	
4	5.91	17.76	34.04	15.5	35.5	
5	6.18	19.24	32.56	14.5	32.5	
6	6.76	17.76	35.52	14.5	30	
7	7.36	16.28	32.56	11.5	31	
8	9.00	16.28	32.56	14	35	
9	9.24	16.28	31.08	18	36.5	
10	7.65	16.28	31.08	17	39	
11	6.80	17.76	32.56	17	36	
12	8.29	17.76	34.04	15	36.5	
13	8.26	17.76	34.04	14	32	
14	11.98	17.76	32.56	12	31	
15	13.47	16.28	31.08	14	31.5	
16	14.89	16.28	31.08	12	32.5	
17	12.81	17.76	31.08	16.5	42	
18	9.22	16.28	32.56	16	41.5	
19	7.60	17.76	34.04	14	32	
20	9.89	17.76	31.08	15	32	
21	12.95	17.76	31.08	17	34.5	
22	22.21	17.76	32.56	15.5	36	
23	24.39	17.76	32.56	14.5	38	
24	21.39	17.76	31.08	14	41	
25	14.14	16.28	31.08	15	41.5	
26	10.76	16.28	31.08	17	43.5	
27	6.54	19.24	35.52	11.5	34	
28	4.95	19.24	34.04	11	27	Blind spot
29	15.32	17.76	32.56	15	36	
30	27.69	17.76	32.56	14.5	36.5	
31	55.84	17.76	32.56	16	44.5	Central area
32	30.56	16.28	31.08	17	43.5	
33	21.45	17.76	31.08	15.5	46.5	
34	12.30	16.28	29.6	15.5	47	
35	9.41	16.28	31.08	22	43.5	
36	6.08	19.24	35.52	15	32.5	
37	10.37	17.76	32.56	14	33	
38	17.78	17.76	34.04	14	34.5	
39	25.42	17.76	34.04	14	34.5	
40	30.01	17.76	31.08	14	40.5	
41	20.43	16.28	31.08	14.5	37.5	
42	14.31	16.28	29.6	17.5	43	
43	11.26	16.28	31.08	15.5	41.5	
44	8.65	17.76	34.04	13.5	36.5	
45	12.31	17.76	32.56	18	42	
46	14.88	17.76	32.56	14.5	37.5	
47	16.92	17.76	32.56	13.5	36	
48	14.88	17.76	32.56	16	33	
49	12.83	17.76	31.08	15	42	
50	9.81	17.76	32.56	17.5	43	
51	8.95	17.76	34.04	17	42	
52	10.98	17.76	31.08	16.5	36.5	
53	11.63	17.76	32.56	14	33	
54	12.72	17.76	31.08	15.5	38	
55	12.15	17.76	31.08	16	39.5	
56	10.28	17.76	31.08	17.5	40.5	
57	8.02	16.28	34.04	15.5	36.5	
58	7.94	16.28	32.56	16	38.5	
59	9.29	17.76	31.08	18.5	38	
60	6.17	17.76	32.56	14.5	42	
61	9.04	17.76	32.56	19	47.5	