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Flicker electroretinogram in retinitis pigmentosa

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Abstract. Electroretinograms (ERGs) were recorded as a function of flicker frequency from 5 to 50 Hz for 14 retinitis pigmentosa (RP) patients, 12 normal subjects and 1 rod monochromat. Data were analyzed by measuring the angular position of the response maximum, i.e. the phase, as a function of pulse-train frequency. Flicker ERGs obtained from the RP patients showed non-linear, frequency-dependent phase shifts when compared to the normal data. These phase shifts were simulated in a normal observer by attenuating the stimulus luminance by 1 log unit. However, the shape of the waveforms recorded from the normal differed markedly from those recorded from the RP patients. The differences, but not the ratios of the times-to-peak of the positive and negative ERG wavelets were longer in the RP patients than in the normal. These data suggest that the temporal anomalies in the RP flicker ERG are most likely due to changes in the amplitudes and time constants of the ERG components, and not simply to a reduced quantum catch or photoreceptor loss.

Introduction

Following the seminal work of Berson and his colleagues (Berson et al., 1968; 1969; 1969a; 1969b; Berson and Kanters, 1970) several investigators have examined changes that occur in the temporal characteristics of the electroretinogram (ERG) of patients with primary retinitis pigmentosa (RP) (Marmor, 1979; Anderson et al., 1979; Rothberg et al., 1982). Although the previously reported delays in the implicit-time of the single flash ERG b-wave of RP patients are difficult to replicate (Massof et al., 1984), temporal anomalies in the flicker ERG have been seen consistently (Marmor, 1979; Anderson et al., 1979; Rothberg et al., 1982). Past studies have characterized these flicker ERG temporal anomalies as 'implicit-time' delays; although, with the exception of Anderson et al. (1979) the presentation of results has been largely qualitative and generally based on only one flicker frequency (usually 30 Hz).

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Anderson et al. (1979) employed sine-wave flicker, instead of the usual train of pulses, and, following Fourier analysis, presented their data on polar coordinates as amplitude versus phase of the fundamental Fourier component. At 24 Hz there appears to be an orderly clockwise spiral into the origin for the RP patients, with phases spanning 180°. This result implies an inverse correlation between cone ERG amplitude and implicit time. However, for other frequencies, particularly 32 Hz, the RP phases appear to cluster at values 130° to 160° from the normal cluster, or in some cases fall at the normal phase despite decreases in amplitude.

Fricker (1971) examined the relationship between flicker ERG phase and flicker frequency in three RP patients, employing a synchronous detector technique. For one young patient, whose diagnosis was equivocal, Fricker found a steeper than normal phase versus fequency function for frequencies less than 40 Hz. Above 40 Hz the function was shallower than normal. These results indicate there is an ERG response delay in this patient, relative to normal, for the lower flicker frequencies. At the higher flicker frequencies, the shallower function corresponds to a faster than normal response for the patient. This two-branched phase versus frequency function contradicts the notion of a simple delay in the ERG. Indeed, Fricker's second RP patient exhibited only the shallower branch of the ERG phase versus frequency function. For that RP patient, we would conclude that the ERG latency is faster than normal.

At present, we do not understand the underlying abnormalities responsible for the temporal anomalies seen in the RP flicker ERG. Because the flicker ERG is a patently nonlinear signal, i.e. sine wave flicker does not produce a sinusoidal response (Troelstra and Garcia, 1975), we cannot necessarily conclude that for RP patients there are simple latency changes or changes of rates within the ERG generators. To further study temporal abnormalities in the RP ERG, we extend Fricker's analysis (albeit not his technique) by measuring angular position of the response maximum (i.e. phase) as a function of pulse-train frequency. We find a nonlinear, frequency-dependent phase-shift in all RP patients. These temporal anomalies are accompanied by a change in the shape of the ERG waveform.

Methods

Subjects

Flicker ERGs were recorded from twelve ophthalmologically normal subjects ranging in age from 13 to 45 years old (all subjects were less than 5 diopters myopic), from fourteen patients diagnosed as typical retinitis pigmentosa (RP), and from one rod monochromat. The clinical characteristics of the patients are listed in Table 1. Informed consent was obtained from each participant in the study.

Table 1. Summary of patient characteristics

Typical RP*	Sex	Age	Genetic subtype**	Visual field area***	Functional subtype****
1	F	29	S	42.4	2
2	F	16	S	37.1	2
3	M	41	S	12.1	2
4	F	35	S	34.5	2
5	F	39	D	40.9	2
6	M	13	S	50.5	2
7	F	38	S	36.9	2
8	F	45	S	49.7	2
9	M	12	D	39.4	1
10	F	36	S	25.9	2
11	F	20	D	45.8	2
12	F	26	D	45.3	2
13	M	42	D	32.8	2
14	F	28	M	40.6	2

*All patients met the clinical criteria for typical RP: (1) nightblindness; (2) midperipheral ring-like scotoma or contracted visual field; (3) good visual acuity until late in the course of the disease; (4) narrowed retinal arterioles; (5) intraretinal, midperipheral, bone-spicule-like pigmentation; (6) vitreous degeneration; and (7) no known systemic, metabolic, inflammatory, toxic, traumatic, or dietary cause of the retinal degeneration.
**D = dominant, S = simplex (no other family history), M = multiplex (affected siblings only).
***Visual field areas are for the Goldmann V/4e target and are expressed in visual field units (vfu). One vfu is the area of a circular visual field of 10° radius. A normal visual field $\geq$ 45 vfu.
****Type 1 RP is characterized by an early and diffuse loss of rod function with a later, regionalized, progressive loss of cone function. Type 2 RP is characterized by a relatively late onset, regionalized, progressive, combined loss of rod and cone function (Massof and Finkelstein, 1979).

Apparatus

The ERG was recorded in response to trains of 10 μsec, 5.5 cd-sec/m^2 (maximum luminance for a single flash) ganzfeld white light flashes from a xenon discharge tube (Grass PS-22 photostimulator, illuminating the interior of a 50 cm diameter ganzfeld diffusing sphere). Flash train frequency varied from 5 Hz to 60 Hz in 5 Hz increments.

The ERG was recorded with a Burian-Allen bipolar contact lens electrode wetted with a methylcellulose solution. The signal was amplified (0.1 to 300 Hz band-pass) and displayed on a storage oscilloscope. The displayed ERG and stimulus calibration marks were photographed, and all measurements were made from the photographic prints.

Procedure

The subjects' or patients' pupils were dilated with a combination of 1% tropicamide and 10% phenylephrine hydrochloride; the cornea was anesthetized with 0.5% proparacaine hydrochloride. Recordings were made from only one eye; the fellow eye was occluded. The measurements described here followed 10 min of dark-adaptation.

Flickering stimuli, ranging from 5 Hz to 60 Hz, were presented to the subject or patient at maximum stimulus intensity, beginning with the lowest frequency and making successive measures at 5 Hz increments. At each frequency the flickering stimulus was presented continuously until amplitude and phase variations were eliminated (i.e. four successive records could be superimposed), at which time the ERG responses were photographed. Thus, we presume adaptation level was stable, although not necessarily the same for all flicker frequencies.*

Results

Typical normal flicker ERG waveforms are illustrated in Figure 1 for the indicated frequencies. Responses for frequencies ranging from 5 Hz to 20 Hz have complex shapes with multiple minima and maxima. At frequencies of 25 Hz and above, the responses are roughly sinusoidal.

The ERG in response to 5 Hz flicker is characterized by an initial corneo-negative potential (presumably an a-wave), followed by a major corneo-positive potential (presumably a b-wave). Other minor wavelets occur throughout the potential. The first minimum occurs 16 msec after the beginning of the waveform (i.e. the first systematic departure from baseline), the maximum occurs at 33 msec, and the slow positive rise after the last wavelet begins at 83 msec. Thus, for frequencies up to 12 Hz the total waveform duration (83 msec) is shorter than one period of the flicker; up to 25 Hz the duration for the major components of the waveform (33 msec) is shorter than one period of the flicker; above 25 Hz the flicker periods are shorter than the time course of the major positive and negative components.

Figure 2 (left) illustrates flicker ERG waveforms from the rod monochromat at 5 Hz, 7.5 Hz and 10 Hz. At 5 Hz the major positive peak occurs at 50 msec. This peak corresponds in time to the minor wavelet (right) occurring after the major positive peak for the normal 5 Hz flicker ERG. The rod monochromat's flicker ERG amplitude is 25% of the normal flicker ERG amplitude at 5 Hz. At 7.5 Hz the amplitude is markedly reduced for the rod monochromat and flicker fusion occurs by 10 Hz. Generalizing from these data, we may conclude that with the luminance used in this study, the rod system

*By using 10 μsec pulses, duty-cycle is frequency-dependent, consequently average luminance would be expected to increase with flicker frequency. However, the level to which the driving capacitors in the stimulator can recharge between pulses also depends upon flicker frequency, resulting in a decrease in pulse amplitude with increasing frequency. These two uncontrolled parameters partially offset each other in the determination of adaptation level. A preferable method would be to use square-wave flicker of constant average luminance, as done by Anderson et al. (1979). However, most clinical studies and most clinical ERG laboratories employ the pulse-train stimulus for eliciting the flicker ERG. Thus, in order to be consonant with previous studies of flicker ERG temporal abnormalities in RP and to collect data with a procedure that is easily replicated in other clinical laboratories, we have chosen the usual clinical stimulus for this study of the RP flicker ERG.

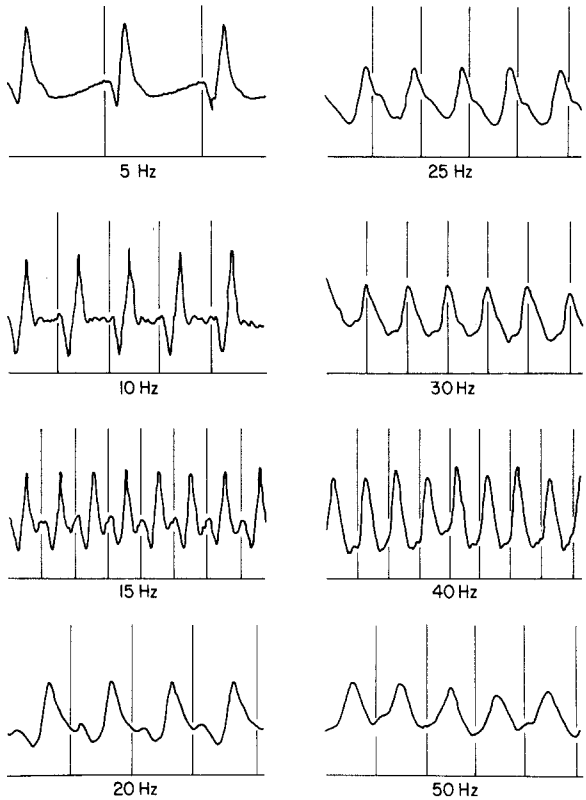

Figure 1. ERG waveforms recorded as a function of flicker frequency from a normal subject. Vertical lines indicate time of stimulus presentation.

makes very little contribution to the normal flicker ERG, even at low frequencies.

In angular terms, each period of flicker contains 360°, regardless of flicker frequency. To express the angular position of an ERG waveform component in relation to the stimulus, we converted the measured durations (msec) to phase angles (degrees) by the equation

$$\phi = .36\nu t \tag{1}$$

where t is the time in msec from the flash to the waveform component of interest, ν is the flicker frequency in cycles per second, .36 is the conversion constant in degrees-seconds/cycles-msec, and ϕ is the derived phase angle in degrees.

For all normal and RP patient data, duration in msec from a stimulus mark to the first positive potential was meaured and converted to angular phase by Eq. (1). Phase angles as a function of pulse-train frequency, for all normal subjects, are plotted in Figure 3; note the discontinuity between 30 Hz and

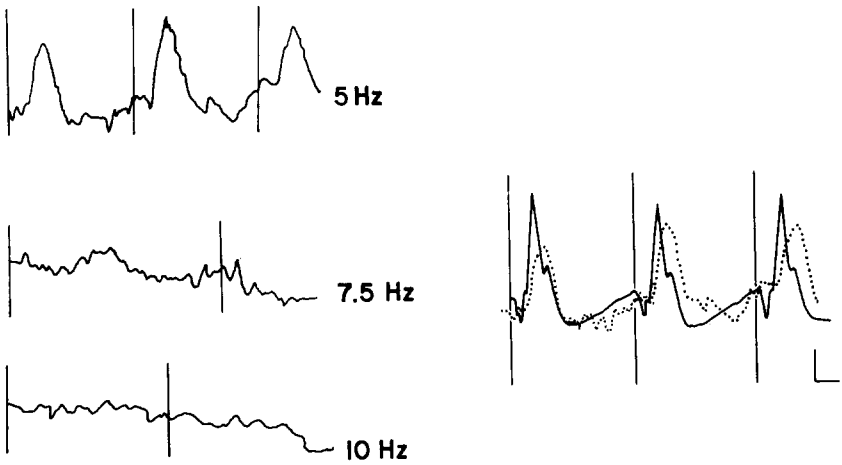

Figure 2. (left) ERG waveforms recorded from a rod monochromat as a function of flicker frequency. (right) ERGs recorded to a 5 Hz flickering stimulus for a rod monochromat (dotted line) superimposed on the 5 Hz response from a normal subject (solid line). Vertical lines indicate time of stimulus presentation. Calibration bars indicate 50 msec horizontally, and 100 μV vertically for the normal waveform and a 50 μV vertically for the rod monochromat.

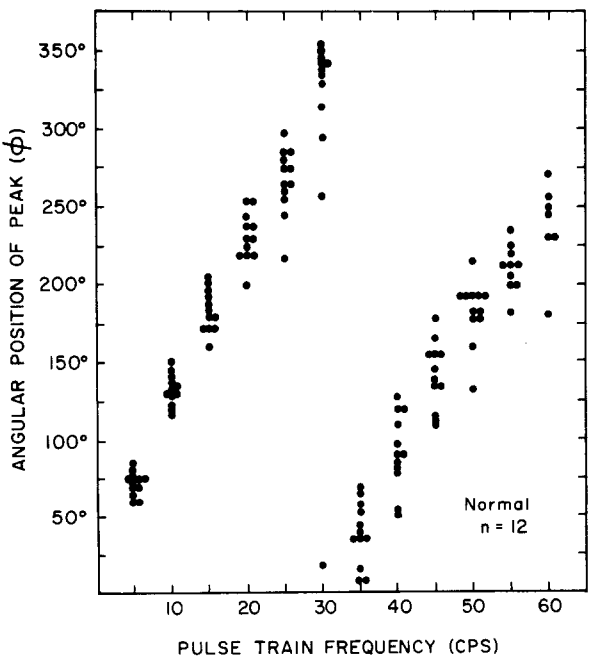

Figure 3. The angular position (ϕ) of the dominant peak of the flicker ERG waveform plotted as a function of flicker frequency for 12 normal subjects.

35 Hz. Figure 4 illustrates phase angles as a function of pulse-train frequency for all the RP patients. The RP functions are steeper and the discontinuity occurs between 20 Hz and 25 Hz.

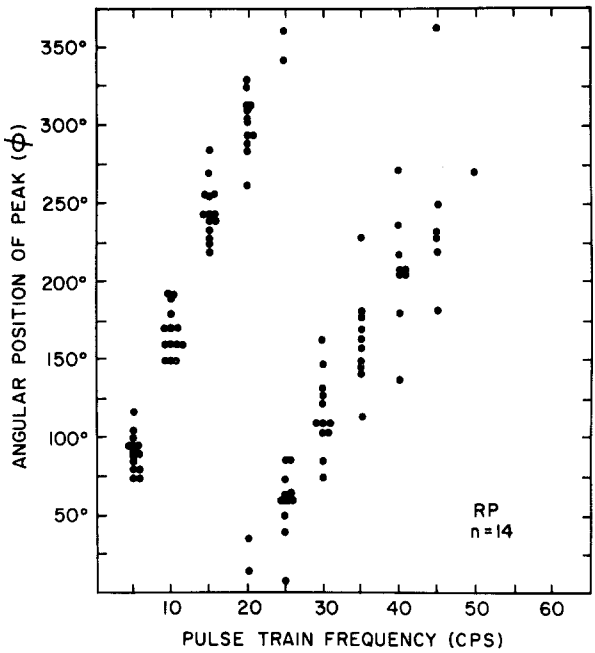

Figure 4. The angular position (ϕ) of the dominant peak of the flicker ERG waveform plotted as a function of flicker frequency for 14 RP patients.

The functions Figures 3 and 4 are made continuous if 360° is added to all high frequency phases after the discontinuity. The resulting continuous functions for normal subjects and RP patients are plotted together in Figure 5. The mean values are connected by straight lines and the error bars denote the range of values for each group. Note that, except for 5 Hz, there is very little overlap between the ranges for the two groups and that the RP function is steeper than the normal function.

Figure 6 illustrates this phase function abnormality as the difference between mean normal and mean RP phase as a function of flicker frequency. If the phase differences from normal could be attributed to a simple delay, the function in Figure 6 would be a straight line with positive slope, the value of which would be determined by the delay. The difference is approximately linear from 5 Hz to 20 Hz with a slope of 4.36 degrees/Hz. This slope translates to an average time-to-peak increase of 11 msec in RP patients. However, above 20 Hz the differences become nonlinear and nonmonotonic. The linear difference up to 20 Hz is not surprising since the period of flicker still exceeds

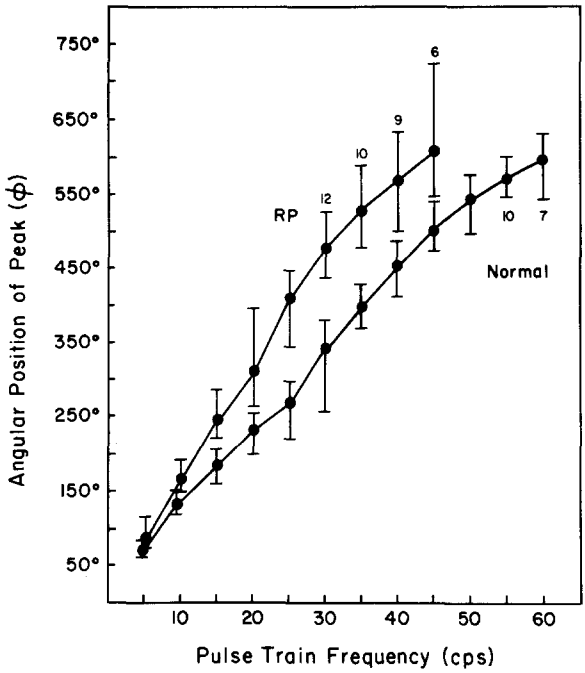

Figure 5. Phase angle (ϕ) as a function of flicker frequency. Filled circles represent the means from 14 RP patients and 12 normal subjects (except where indicated), respectively, and error bars represent the range of values for each group.

the time course of the major components of the ERG; the higher frequency phase differences must be attributed to complex changes in the ERG waveform of the RP patients.

Figure 7 illustrates examples of flicker ERGs at 5 Hz and 25 Hz from a representative normal subject and two representative RP patients. At 5 Hz flicker there appears to be a change in the shape of the ERG waveform of RP patients that may be characterized as a decrease in the amplitude of the corneo-positive component relative to the amplitude of the corneo-negative component. Because these two components will add algebraically with opposite sign, part of the increase in time to peak (11 msec) for RP patients could be due to a simple change in the amplitude ratio of the underlying components. In order to determine if these qualitatively observed shape changes are a generalizable feature of the RP 5 Hz flicker ERG, the ratio of the amplitude of the negative potential, measured from baseline, to the amplitude of the positive potential, measured from the trough of the negative potential, was computed for each RP patient and each normal subject. Histograms of these ratios are illustrated in Figure 8. These results indicate that the average normal positive potential is 1.38 times greater than the average RP positive potential, relative to the amplitude of the negative

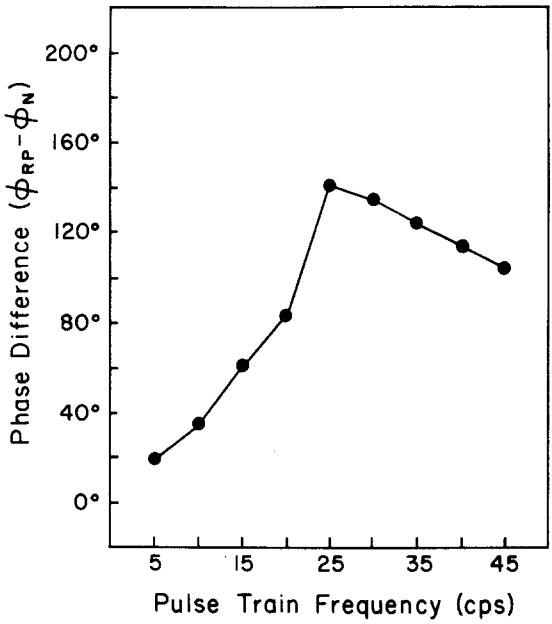

Figure 6. Difference between RP and normal mean phase angles plotted as a function of flicker frequency.

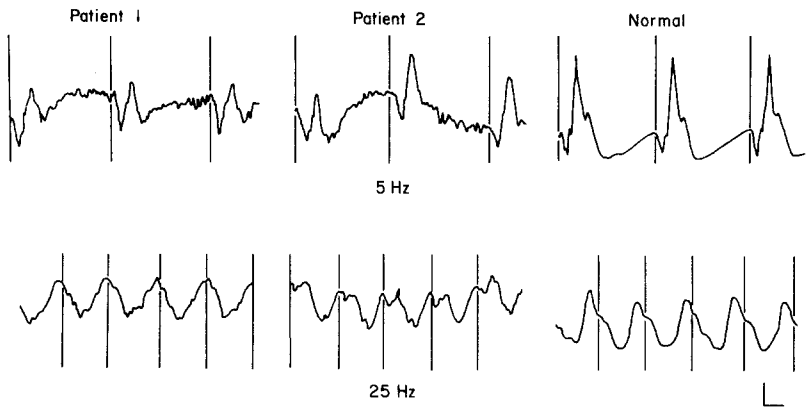

Figure 7. Typical ERG waveforms for 2 RP patients and 1 normal subject in response to a 5 Hz flickering stimulus (top row) and to a 25 Hz flickering stimulus (bottom row). Vertical lines indicate time of stimulus presentation. Calibration bars indicate: (top) 50 msec horizontally, 100 μV vertically for the normal subject and 50 μV vertically for the RP patients: (bottom) 20 msec horizontally, 50 μV vertically for the normal subject and 20 μV vertically for the RP patients.

potential ($t = 1.72$; $df = 24$; $p < .1$ for all data and $t = 2.25$; $df = 22$; $P < .05$ if the normal outlier and RP outlier are excluded).

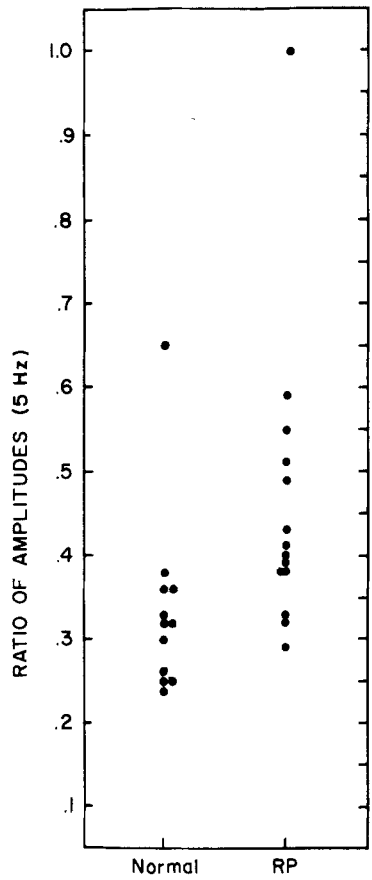

Figure 8. Ratio of the amplitudes of the negative potential to the positive potential of a 5 Hz flicker ERG, for 12 normal subjects and 14 RP patients.

At 25 Hz, the normal flicker ERG is characterized by a major rapidly rising corneo-positive potential (see Figure 7), followed by a shoulder on the descent from the peak. In contrast, some RP patients have a broader-peaked waveform, with no shoulders (patient 1 in Figure 7), and some patients have a double-peaked waveform (patient 2 in Figure 7). Comparison of the waveforms for patients 1 and 2 in Figure 7 suggest the possibility that the broader peak of patient 1 represents a melding of the double-peaks of patient 2. The second peak for patient 2 may correspond to the normal shoulder. Such speculations aside, there clearly are qualitative shape differences between the normal and RP waveforms.

A simple increase in response latency would be expected only to shift the waveform in time; there would be no changes in waveform shape. Such a 'delay'-type of disease mechanisms would require that there be no changes in the rate constants that determine the final waveform shape. On the other

hand, a change in the relevant rate constants may serve to 'smear-out' the waveform in time, causing qualitative shape changes as well as changes in time-to-peak.

To quantify the qualitatively observed waveform shape changes at 5 Hz and 25 Hz, we measured the time from the stimulus flash to the first negative trough and to the succeeding positive peak in all normal and RP records. If the shape of the RP waveform were the same as the normal waveform, the difference between the time to peak and time to trough would be the same for the normal subjects and RP patients. Figures 9a and 9b illustrate histograms of these time differences for normal subjects and RP patients. There is overlap between the two distributions; however, the means are separated by about 4 msec ($t = 2.49$; $df = 24$; $p < .05$ for 5 Hz, $t = 2.64$; $df = 23$; $p < .02$ for 25 Hz) for both flicker frequencies. These results indicate that there is a larger delay in RP patients for the positive peak than there is for the negative trough. Thus, the abnormality in the RP flicker ERG waveshape is consistent with a putative 'smearing-out' of the waveform.

A 'smearing-out' in time of the flicker ERG waveform could correspond to a change in retinal neural-response time-constants. Alterations of these time-constants would affect the differences between times to peaks (Figure 9), however, it would not necessarily affect the ratios of the times to peaks. Figures 10a and b are histograms of the ratio of times to the negative trough to the times to the positive peak for normal subjects and RP patients. For both 5 Hz and 25 Hz, there is no significant difference between the distributions for normal subjects and RP patients ($t = .209$; $df = 24$ for 5 Hz and $t = .682$; $df = 23$ for 25 Hz). This finding is consistent with the hypothesis of a change in waveform time-constants for the RP flicker ERG.

In normal vision, slower time constants are known to be characteristic of lower adaptation levels. With this fact in mind, the simplest explanation for the RP flicker ERG phase shifts would be an abnormally low adaptation level secondary to a reduced quantum catch, perhaps because of shortened photoreceptor outer segments (re Szamier et al., 1979; Sandberg et al., 1981). Figure 11 illustrates flicker ERG phase functions obtained from a normal observer at maximum stimulus luminance (0.0) and at decreasing luminances ranging from 0.6 to 3.0 log units below the maximum. These results indicate that a decrease in luminance serves to shift the normal ERG flicker phase function toward, and past the flicker phase function of the RP patients.

Although the change in the normal flicker ERG phase function with a decrease in luminance is in qualitative agreement with the findings in RP patients, the actual flicker ERG waveforms are not. This difference can be seen in Figure 12 which illustrates normal flicker ERGs for 5 Hz flicker at maximum luminance and at one log unit below the maximum luminance, along with an RP flicker ERG for the same frequency at maximum luminance. Note that for the lower luminance level, the normal flicker ERG exhibits a smaller a-wave relative to the b-wave. This change is opposite that seen for

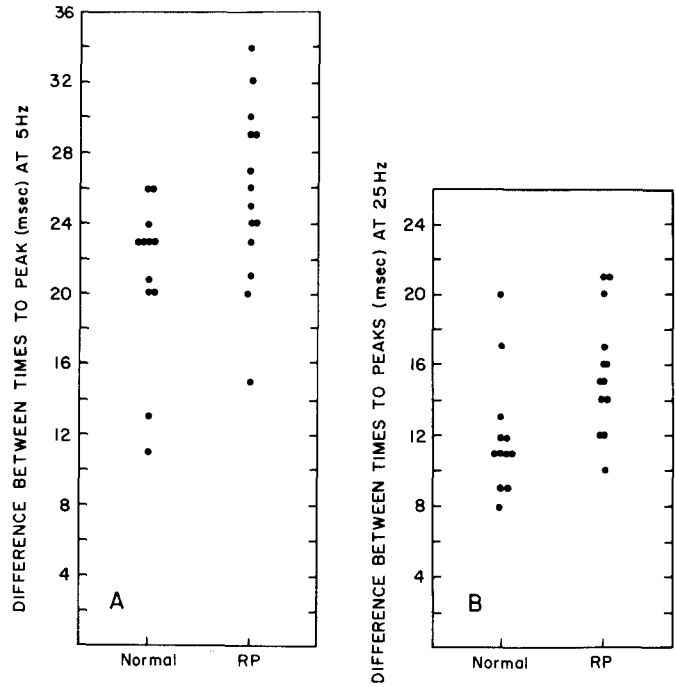

Figure 9. (a) Differences between the times to the first negative trough and the first positive peak of a 5 Hz flicker ERG measured for 12 normal subjects and 14 RP patients. (b) Same as a, except for a 25 Hz flickering stimulus. Data from 13 RP patients were included in this analysis.

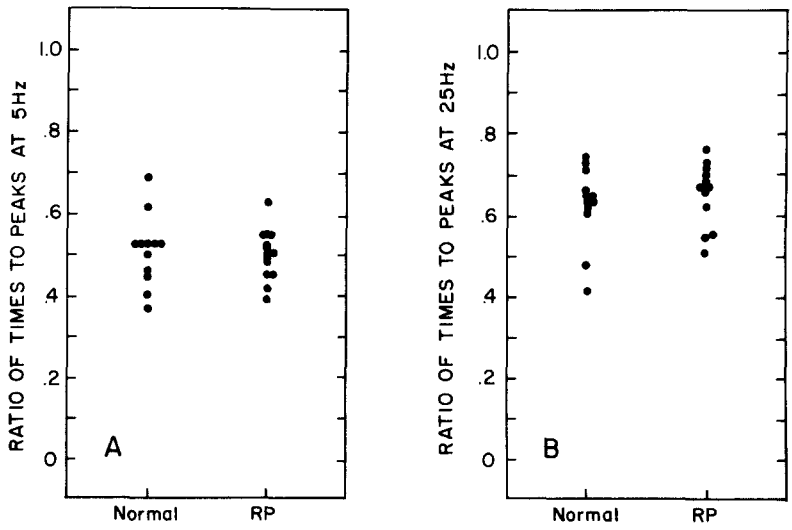

Figure 10. Ratios of the time to the negative trough to the time to the positive peak for 12 normals and 14 RP patients. (a) 5 Hz flicker. (b) 25 Hz flicker. Data from 13 RP patients are shown.

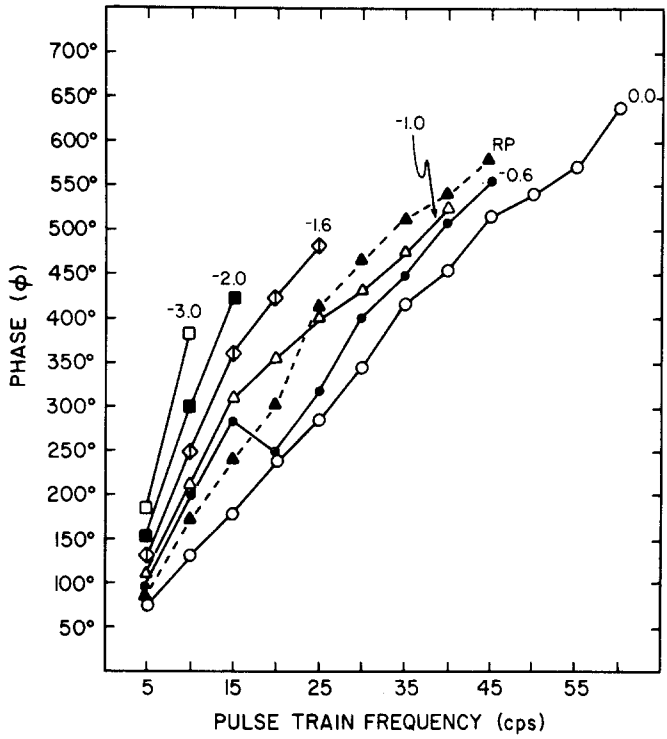

Figure 11. Flicker ERG phase functions plotted for a normal observer as a function of stimulus luminance (solid lines), along with the mean RP flicker phase function for the maximum stimulus intensity (filled triangles).

RP flicker ERGs, where the a-wave is larger relative to the b-wave (i.e. Figure 8). Thus the phase shift seen in the RP flicker ERG most likely cannot be attributed to a lower effective stimulus luminance from reduced retinal sensitivity in RP.

Discussion

Typical RP can be subclassified along genetic lines and according to rod sensitivity loss relative to cone sensitivity loss (re Massof and Finkelstein, 1979). Although early studies suggested that the delay in the flicker ERG could be used to segregate genetic subtypes of RP (Berson et al., 1969b); such observations could not be replicated in later studies (Marmor, 1979; Rothberg et al., 1982). The patients used in the present study represented a mixture of genetic categories. In terms of retinal function, one of the 14 patients was type 1, all others were type 2. Although it was not the purpose of this paper to compare subtypes, the same observations were made in all patients.

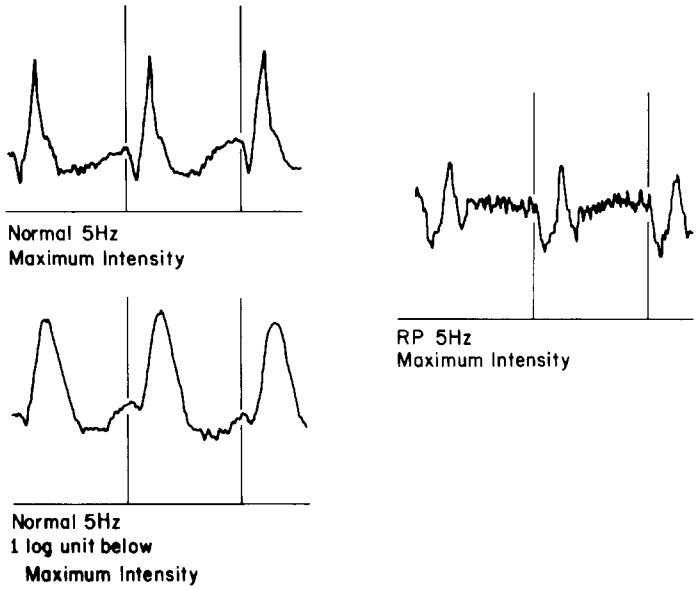

Figure 12. (left) Flicker ERG waveforms, recorded at 5 Hz, from a normal subject at maximum stimulus intensity (top) and at 1 log unit below maximum stimulus intensity (bottom). (right) 5 Hz flicker ERG waveforms recorded at maximum stimulus intensity from an RP patient.

In summary, through the use of pulse-trains varying in frequency from 5 Hz to 60 Hz, the present study found: (1) there is a non-linear, non-monotonic, frequency-dependent phase-shift in the flicker ERG of RP patients in comparison to the normal flicker ERG; (2) the RP flicker ERG waveform is different in shape from the normal flicker ERG waveform; (3) the average time-difference between the positive peaks of the normal and RP flicker ERGs is 4 msec greater than the average time-difference between the negative troughs; (4) the ratio of time to negative trough to time to positive peak is the same for normal and RP flicker ERGs. These results indicate that there are qualitative changes in the RP flicker ERG waveform that may be attributed partially to changes in relative amplitudes and partially to changes in time constants of underlying ERG components. Reducing stimulus intensity produces changes in the normal ERG flicker phase function that appear similar to the RP function. However, the RP flicker ERG waveform is markedly different from the normal flicker ERG waveform measured at lower stimulus intensities. These findings suggest that the temporal anomalies in the flicker ERG of RP patients are more likely due to changes in the response characteristics of the ERG generators than simply to reduced quantum catch from visual pigment loss.

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