Pergamon

PII: S0042-6989(97)00143-0

Vision Res., Vol. 38, No. 1, pp. 163–179, 1998

Printed in Great Britain
0042-6989/98 $19.00 + 0.00

Assessment of Local Retinal Function in Patients with Retinitis Pigmentosa Using the Multi-focal ERG Technique

DONALD C. HOOD,*§ KAREN HOLOPIGIAN,† VIVIENNE GREENSTEIN,† WILLIAM SEIPLE,† JU LI,* ERICH E. SUTTER,‡ RONALD E. CARR†

Received 18 October 1996; in revised form 17 February 1997

To assess local retinal function in patients with retinitis pigmentosa (RP), multi-focal ERGs and local thresholds (static visual fields) were obtained on eight RP patients with visual acuities of 20/25 or better. All eight patients showed multi-focal responses with normal timing within the central 5 deg. However, there were few responses with normal timing in the areas outside the central 7.5 deg, except in the case of the only patient with a 30 Hz full-field response with normal timing. Since full-field ERGs are dominated by responses from the periphery, this finding supplies a foundation for the commonly observed delays in the full-field cone ERGs of patients with RP. With respect to amplitude, only two patients showed multi-focal responses with near normal amplitudes anywhere in the field. The loss of amplitude at any point was not a good predictor of visual sensitivity in the Humphrey visual field. On the other hand, all areas with normal timing had near normal sensitivity. Timing changes appear to be an early indication of local retinal damage to the cone system. Nearly all areas with sensitivity losses greater than 0.5 log unit, and some areas with near normal sensitivity, showed significantly delayed multi-focal ERGs. Finally areas with extreme sensitivity loss show multi-focal responses with a wide range of amplitudes and implicit times across patients, suggesting different mechanisms of disease action in different patients. © 1997 Elsevier Science Ltd

Retinal disease Retinitis pigmentosa Electroretinogram Multi-focal ERG Human

INTRODUCTION

Patients with retinitis pigmentosa (RP) show full-field, cone ERGs that are depressed in amplitude and delayed in timing. The implicit time of the b-wave of the cone ERG appears particularly sensitive and delays can be observed even during the early stages of RP (e.g. Berson, Gouras, Gunkel, & Myrianthopoulos, 1969b; Berson, Gouras, & Hoff, 1969a; Berson & Kanters, 1970; Massof, Johnson, Sunness, Perry, & Finkelstein, 1986; see also Berson, 1993 for a review). Damage to the cone system can also be seen in the patients' photopic, visual fields measured with static perimetry. These visual fields show a range of losses in sensitivity both within and across patients (e.g. Arden *et al.*, 1983; Yagasaki, Jacobson, Apathy, & Knighton, 1988; Massof, Wu, Finkelstein, Perry, Starr, & Johnson, 1984; Nusinowitz & Birch, 1997). However, because the full-field ERG is a summed response from the entire retina, relatively little is known about the relationship between local retinal damage and the delays in the full-field ERG or the losses in the visual fields. Here we assess the electrical activity of local retinal regions using the multi-focal ERG technique.

The focal ERG refers to an ERG elicited with localized retinal stimulation. Typically, the focal cone ERG is obtained with a flickering stimulus train and a light 4 to 15 deg in diameter (e.g. Sandberg & Ariel, 1977; Seiple, Siegel, Carr, & Mayron, 1986; Miyake, Shiroyama, Ota, & Horiguchi, 1988). This technique has proven useful in examining the response from the macula, a response that is obscured in the full-field ERG. For example, the timing of the focal ERG from the central 10 deg can be normal in patients with RP even when their full-field cone ERGs show delays (Sandberg, Jacobson, & Berson, 1979; Sandberg, Effron, & Berson, 1978; Seiple *et al.*, 1986). However, the time involved in obtaining focal ERGs precludes studying more than a few retinal locations in a session.

A recent technique developed by Sutter and colleagues (Sutter, 1991; Sutter & Tran, 1992; Bearse & Sutter,

*Department of Psychology, Columbia University, 406 Schermerhorn Hall, New York, NY 10027, U.S.A.
†Department of Ophthalmology, NYU Medical Center, New York, U.S.A.
‡Smith-Kettlewell Eye Research Institute, San Francisco, California, U.S.A.
§To whom all correspondence should be addressed. [*Fax*: +1-212-854-3609; *Email* don@psych.columbia.edu].

Table 1.

Patient	P1	P2	P3	P4	P5	P6	P7	P8
Category	AR	AR	Ushers II	Simplex	AD	Ushers II	Simplex	Simplex
Age/Sex	45/F	31/F	25/M	39/M	48/F	41/M	51/F	36/M
Visual acuity	20/20+	20/20	20/20	20/20	20/20	20/20	20/16	20/25
30 Hz* Implicit Time	28.8	40.8	39.1	35.6	37.6	39.1	42	nondetect
30 Hz† amplitude	45.6	11.4	3.6	18.6	98.4	41.1	6.8	nondetect
Goldmann V4e								
min	12 deg	25 deg	25 deg	30 deg	30 deg	20 deg	10 deg	18 deg
max	70 deg	68 deg	45 deg	70 deg	75 deg	80 deg	15 deg	55 deg

*Mean normal time: 27.9 msec ± 1.6.
†Mean normal amplitude: 138.6 μV ± 35.2.

1996) appears to overcome this limitation. In this procedure, many retinal areas (103 in this study) are simultaneously, but independently, stimulated and the local ERG contributions are extracted from a continuous ERG recording using cross-correlation techniques. Within a single brief (4–16 minute) recording, 103 focal ERGs are obtained. These individual focal responses have a biphasic waveform with a negative potential followed by a positive potential. These biphasic responses appear to be generated by the same cells generating the a-wave and positive peaks of the full-field cone ERG (Hood, Seiple, Holopigian, & Greenstein, 1997).

Recently, multi-focal ERGs in patients with RP have been reported to be reduced in amplitude in regions of visual field losses (e.g. Kondo, Miyake, Horiguchi, Suzuki, & Tanikawa, 1995; Seeliger, Kretschmann, Ruther, Apfelstedt-Sylla, & Zrenner, 1996; Hood, Holopigian, Greenstein, Seiple, Sutter, & Carr, 1996). However, it is not clear how the timing changes seen in the full-field ERG are related to the timing of local retinal areas. Further, the relationship between local sensitivity changes and multi-focal ERG changes has yet to be explored. In this paper, we assess local retinal function in eight patients with RP who have good central vision. In experiment 1, the amplitude and timing of the patient's multi-focal ERGs are compared with those of a group of control subjects. In experiment 2, the patients' multi-focal ERGs are compared with local sensitivity measured with Humphrey visual fields.

EXPERIMENT 1

Methods

Patients. Eight patients were recruited from the private practice of one of the authors (R. E. Carr). Retinitis pigmentosa was diagnosed based upon funduscopic findings, elevated dark-adapted thresholds, constricted visual fields, and severely reduced full-field ERGs. The criteria for inclusion included corrected visual acuity of 20/25 or better and central Goldmann visual fields (V4e) of 10 deg or greater. Patients ranged in age from 25 to 51 years and had no other ocular or systemic abnormalities. Summary information can be found in Table 1. The eye with the best visual acuity was tested. If the visual acuity was the same in both eyes, then the right eye was tested. In all but one case (P8), this resulted in the testing of the right eye. For all quantitative comparisons of both the multi-focal and visual fields, the records of P8 were reversed so that comparable parts of the retina were being compared.

Control subjects. Four control subjects of comparable ages [21 (male), 37 (female), 52 (female), and 53 (male) years] to the patients participated in the study. All had normal color vision, normal full-field ERGs and normal ophthalmologic examinations.

Patients and control subjects signed informed consent forms after the experimental procedures were described to them. Tenets of the Declaration of Helsinki were followed and institutional human experimentation committee approval was obtained.

The multi-focal technique. The multi-focal technique is briefly described below; a more complete description can be found in the literature (e.g. Sutter, 1991; Sutter & Tran, 1992; Wu & Sutter, 1995; Bearse & Sutter, 1996). Figure 1(A) shows the spatial paradigm used in the current study. The subject fixates on the center of a display of 103 hexagons which fall within an area with a diameter of 50 deg. The sizes of the hexagons are scaled to produce approximately equal amplitude multi-focal responses in control subjects (Sutter & Tran, 1992). During stimulation the subject sees a field of 103 flickering hexagons. Each hexagon has a probability of 0.5 of being white or black on each frame. The frame is changed every 13.33 msec (a frame rate of 75 Hz). Each hexagon in the array is stimulated with the same sequence of white and black, but this sequence is lagged by different amounts for each location. When the lags are much greater than the duration of the local responses, the responses associated with the individual hexagons are effectively uncorrelated. The local response is computed as the cross correlation between the sequence and the continuously recorded ERG. The waveforms in Fig. 1(B) are the resulting 103 focal responses for a control subject. These are first-order components and can be thought of as the average response from a particular retinal area unaffected by stimulation at any other point in the array or any other point in time. Interactions between a sequence of frames can also be derived as higher-order responses. Under most conditions, including the ones used here, the higher-order components are relatively small [see Sutter & Tran (1992)].

Stimulus conditions. The stimulus array was generated

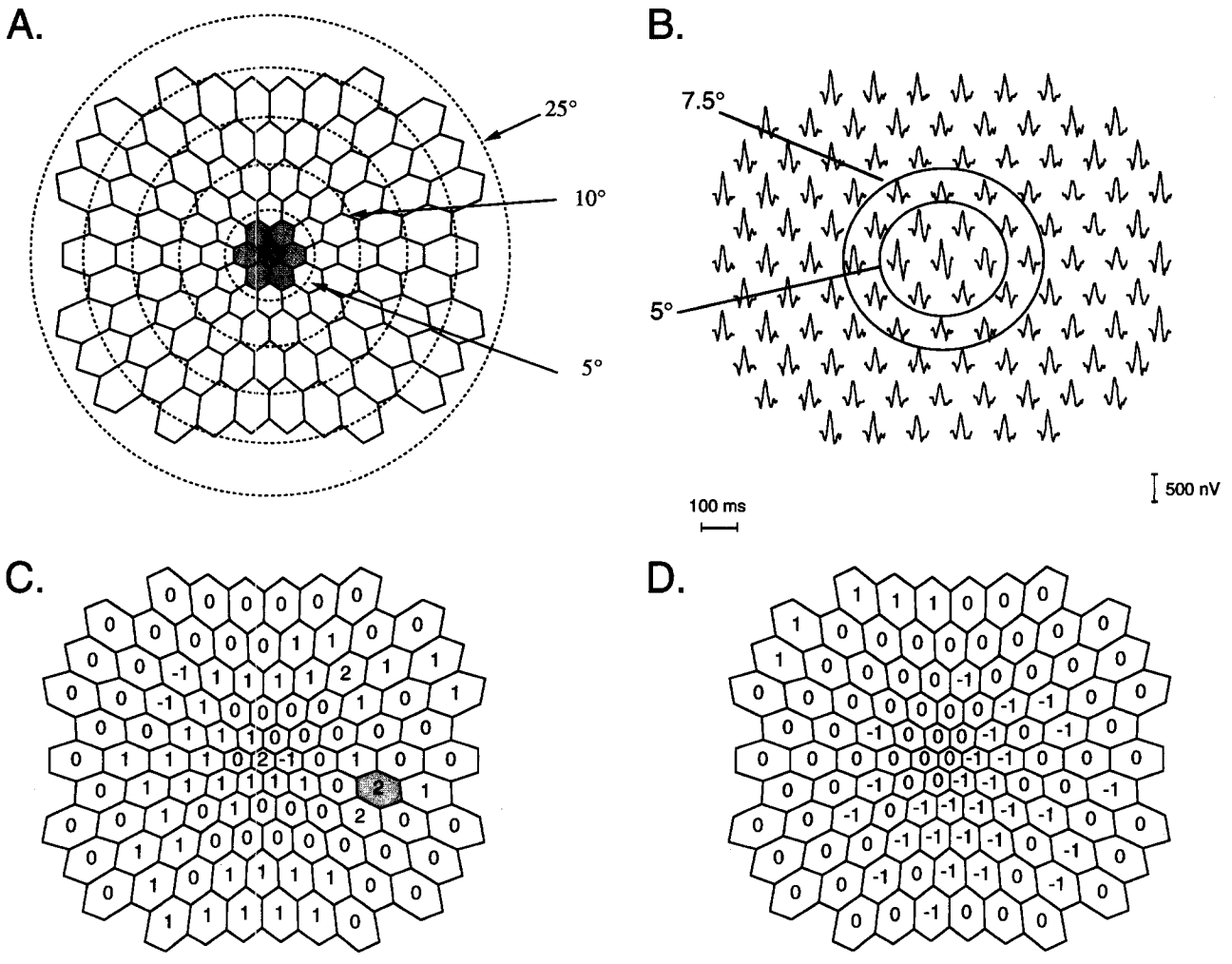

FIGURE 1. (A) The display employed in the multi-focal recordings. Circles with radii as indicated are shown for reference. (B) Multi-focal records are shown for one of the controls. (C) ERG delay field for the records in (B). (D) ERG amplitude loss field for the records in (B).

on a high resolution black and white Dotronix monitor (EM2400-789) by means of a customized Macintosh video card from EDI. The monitor was positioned 32 cm from the subject and the 103 hexagons fell within a field of about 47 deg (width) by 39 deg (height). The white and black hexagons were 400 and 2 cd/m^2, respectively. The area surrounding the array of hexagons was set to 200 cd/m^2 and a central cross was used for fixation.

Recording techniques. One eye was dilated (1% cyclopentolate hydrochloride and 2.5% phenylephrine hydrochloride) and kept light-adapted at room illumination until the experiment began. The diameter of the dilated pupil ranged from 7 to 9 mm across subjects. Responses were obtained from the anesthetized cornea with a bipolar, contact lens electrode (Burian-Allen). Corrective lenses were used to provide the subjects with their best corrected acuity for the viewing distance (32 cm).

To obtain multi-focal ERGs, the continuous ERG record was amplified with the low- and high-frequency cut-offs set at 10 and 300 Hz and was sampled every 0.833 msec (1200 Hz) with an A/D board. [A recent study showed that 10 Hz filtering can distort the waveform of the multi-focal ERG and recommended using a 1 Hz cut-off (Keating, Parks, Williamson, Evans, Jay, & Elliott, 1996). This is particularly troublesome for sustained negative ERGs seen with some retinal problems as it makes them appear biphasic. However, the effect of using a lower cut-off is relatively minor under most conditions, including those of the present study. (See also Fig. 5 in Hood *et al.*, 1997.) In fact, our recordings with a 1 Hz cut-off indicate less than a 1 msec increase in the implicit time of the peak of the normal multi-focal responses. If anything, the increase in the implicit time of the patients' slower responses would be slightly greater and would further enhance the major effect in this paper.]

The m-sequence had 2^{14}-1 elements and required 3.6 min for a single run. [Special care was taken in the selection of the m-sequence so as to avoid potential contamination of the first-order component by higher-order terms (Sutter & Tran, 1992).] To improve the subject's ability to maintain fixation, this 3.6 min period was broken up into eight, overlapping segments each of 27 sec duration (Sutter & Tran, 1992). A single session lasted about 30 min and included four 3.6 min runs. The

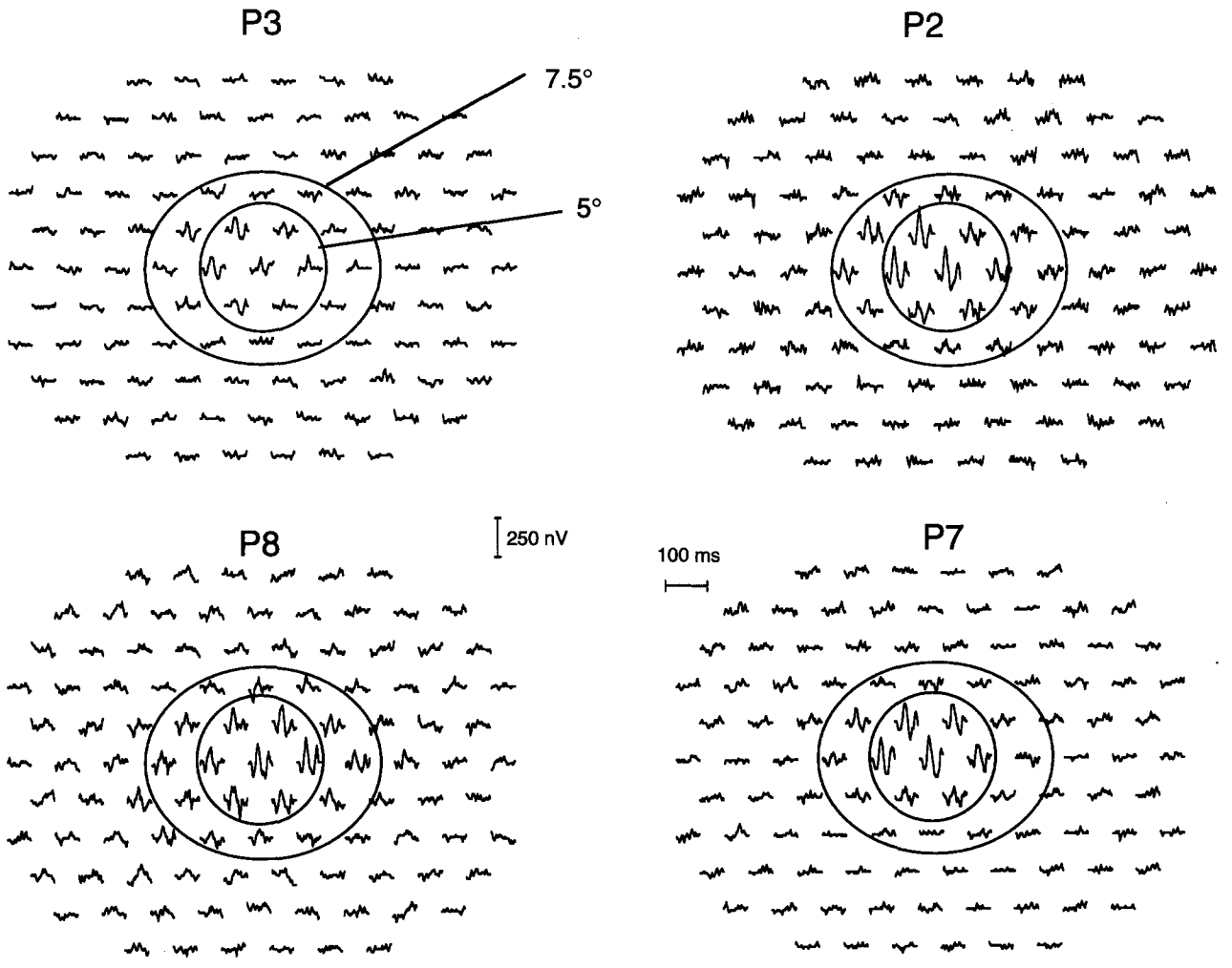

FIGURE 2. The multi-focal records for four of the patients. The circles in each panel indicate the areas with a radius of 5 or 7.5 deg and include the responses to the central 7 and 19 hexagons, respectively. The calibration markers in the center of the figure apply to all records.

data from the four runs were combined to form a single record. Stimulus control as well as the data collection and analysis were performed by the VERIS software from EDI. [See Sutter (1991) and Sutter & Tran (1992) for more details.]

Analysis of individual multi-focal responses. The amplitudes and implicit times of the individual multi-focal responses were measured using a program written in MATLAB. First, the 103 responses were smoothed (equivalent to low pass filtering with a 3 db cut-off at about 100 Hz) and then the initial trough and peak identified. Responses that did not exceed a trough-to-peak amplitude of 90 nV were not considered further. We chose to take a conservative approach to identifying signals (i.e., "true" responses). First, the same criterion trough-to-peak amplitude (90 nV) was used for all subjects; and second, the level of this criterion amplitude was chosen to avoid mistaking noise for signal (i.e., the false alarm rate close to zero)*.

Results

Figure 1(B) shows the 103 multi-focal ERGs from a control subject. The multi-focal ERGs for all eight patients can be found in Fig. 2 and Fig. 3. Note the calibration bars in these figures. The amplification in Fig. 3 is the same as that for the control subject [Fig. 1(B)], while the amplification in Fig. 2 is 2-times greater. The circles shown on all response arrays have a radius of 5 deg (small) or 7.5 deg (large). The patients' multi-focal

*We are faced with a classic signal-to-noise problem. As long as the noise and noise + signal distributions overlap, any criterion will involve a trade-off between the number of misses (failing to identify real signals) and the number of false alarms (identifying noise as a signal). Our approach was to choose a high enough criterion to ensure that the number of "false alarms" would be close to zero. The following analysis suggests that we were successful. As Fig. 5 indicates, P3 has no activity outside of the central 7.5 deg. Using the 90 nV criterion only five responses were identified as signal (see Fig. 6). Decreasing the criterion by 20% to 72 nV resulted in the identification of three additional records in the central 7.5 deg; these are probably correctly identified "signals". But three additional records in the periphery were also identified and these are undoubtedly "false alarms". Further, we analyzed the records of a patient with poor central vision and visual field showing depressed sensitivity across the entire field. His summed multi-focal for the entire field showed no activity. With a criterion of 90 nV, none of the responses were identified as signal. Decreasing the criterion by 20% identified seven responses, which are undoubtedly "false alarms".

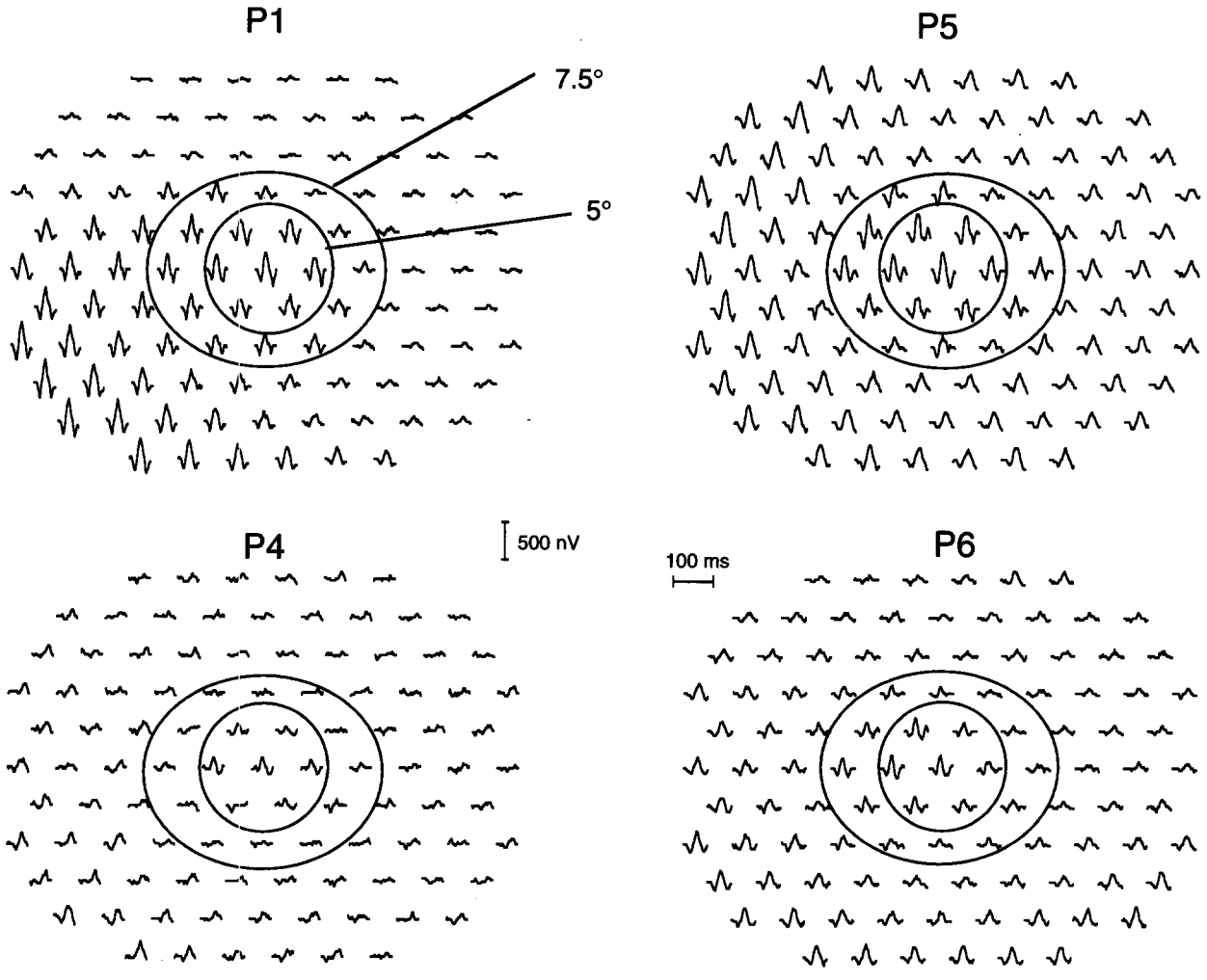

FIGURE 3. The multi-focal records for four of the patients. The circles in each panel indicate the areas with a radius of 5 or 7.5 deg and include the responses to the central 7 and 19 hexagons, respectively. The calibration markers in the center of the figure apply to all records.

ERGs, discussed further below, show a range of amplitudes and waveforms.

Responses from the central 5 deg region. Based upon our selection criteria, all patients had central Goldmann visual fields of greater than 10 deg (V4e target) and visual acuities of 20/25 or better (see Table 1). In fact, seven patients had visual acuities of 20/20 or better. Notice in Figs 2 and 3 that all patients show responses in the central 5 deg (shaded area in Fig. 1(A) and small circles in Figs 2 and 3). Figure 4 compares the summed multi-focal responses of each patient to those of the controls. In particular, the uppermost traces superimposed in Fig. 4 are the sum of all 103 multi-focal responses (Total) or the sum of the central seven responses (Central 5 deg) for each of the four control subjects. To allow for an easier comparison of implicit times, the uppermost traces in the two right panels of Fig. 4 are these same responses shown normalized to have the same peak-to-trough amplitude. The average implicit times of these summed multi-focal ERGs for the control subjects are indicated as the dashed, vertical lines and were 27.8 msec for the total and 28.8 msec for the central 5 deg. This difference in implicit times between central and peripheral multi-focal responses is present in all the normal subjects and has previously been reported in studies of multi-focal ERGs (e.g. Sutter & Tran, 1992) as well as in earlier work with the focal ERG (Sandberg, Effron, & Berson, 1978; Biersdorf, 1982).

Figure 4 also presents the summed responses from the eight patients on the same scale as the control responses (left panels) and normalized (right panels). As expected, the summed Total response is smaller than normal in the patients (left panel). The summed responses from the central 5 deg are also markedly smaller in six of the eight patients (second panel). Given that only one of the eight patients had a 30 Hz full-field ERG with normal timing, the implicit times of the multi-focal ERGs are of particular interest. The implicit times of the full-field 30 Hz flicker responses are shown in parentheses in Fig. 4 (see also Table 1). For one subject (P1), the flicker response had normal timing; for another subject (P8), the flicker response was nondetectable; for the other six patients, the responses were markedly delayed. The patients' summed multi-focal records are arranged in Fig. 4 based upon the implicit time of the Total summed response. Half of the patients had normal implicit times for the Total response. But, all eight had implicit times that were at least as fast as normal for the central 5 deg.

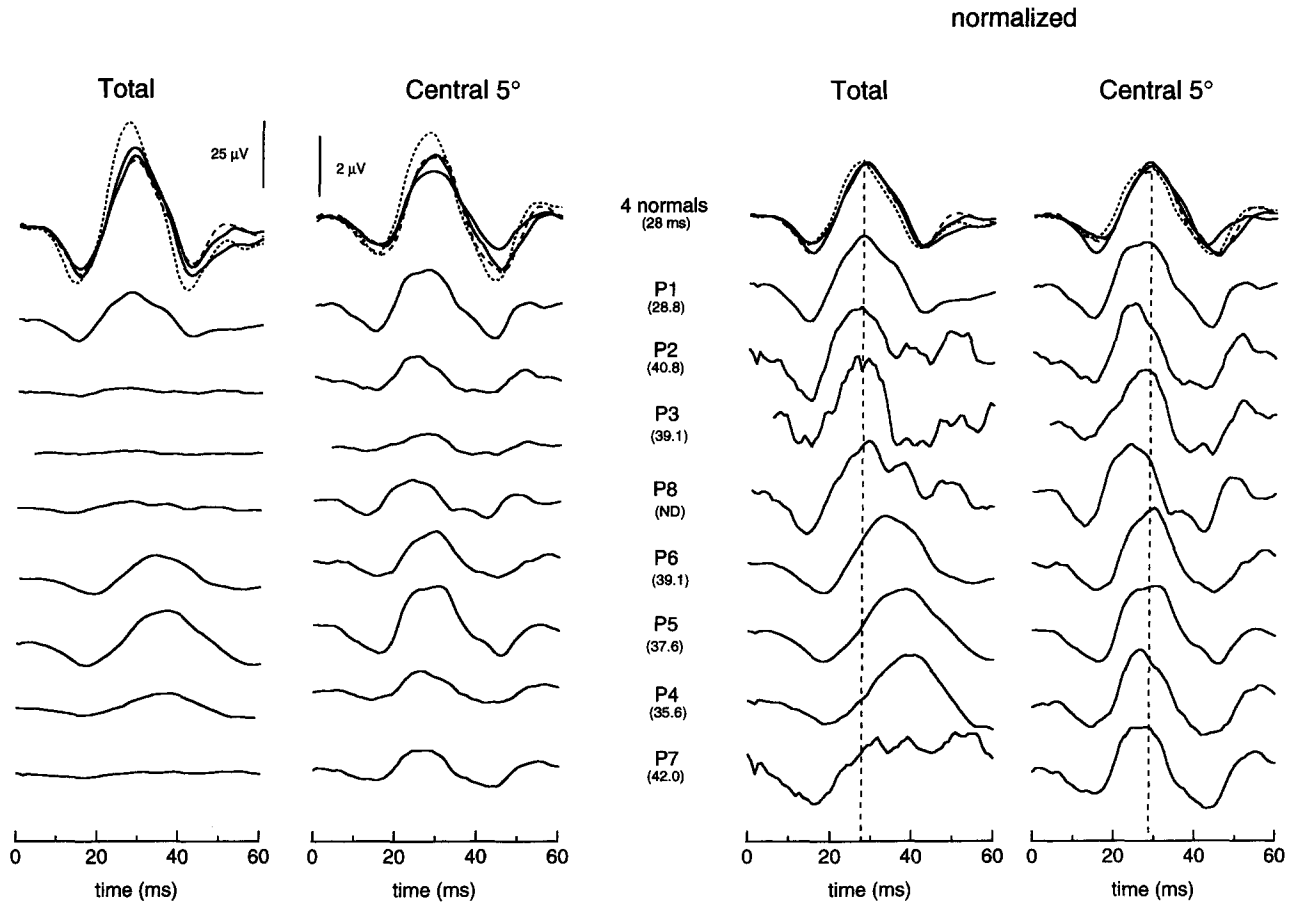

FIGURE 4. The summed multi-focal ERGs for all 103 responses (Total—first panel) and for the central seven responses (Central 5 deg—second panel) are shown for four control subjects and the eight patients with RP. The same records are shown again in the two right-hand panels normalized to the same trough-to-peak amplitude.

The multi-focal response to the central 5 deg is not delayed in this population of patients with good central acuity and delayed full-field ERGs, although the amplitudes are, in general, smaller (Sandberg, Jacobson, & Berson, 1979; Seiple *et al.*, 1986).

Responses from the peripheral region. One surprising aspect of the above analysis is the normal timing of the Total response in patients with markedly delayed full-field flicker ERGs. This apparent contradiction is easily understood when the size of the stimulus [Fig. 1(A)] and the relative contribution of the central regions are considered. The array used in the multi-focal paradigm covers less than one-quarter of the total cones stimulated in the full-field paradigm (Hood *et al.*, 1997). Thus, the full-field ERG will be dominated more by contributions from the periphery than will the Total multi-focal response. The multi-focal display does not include the far periphery, but we can compare the responses to the central hexagons with the responses to the more peripheral hexagons. The larger circle in Fig. 1(B), Figs 2 and 3 has a radius of 7.5 deg and includes the central 19 hexagons; this region is called the "central 7.5 deg" in Fig. 5. A "peripheral region" was defined as the 84 hexagons outside the central 7.5 deg. Most of the patients have clearly detectable signals in most of the locations inside the central 7.5 deg. However, there is considerable variation in the size of the responses in the peripheral region, both across and within subjects.

Figure 5 allows a closer look at the summed responses from the peripheral region. In each panel, three summed multi-focal responses are shown for a single patient. The response in bold and labeled "peripheral" is the sum of the 84 responses outside the central 7.5 deg. The summed response for the central 7.5 deg (the central 19 hexagons within the large circle) and the Total response are also shown. The Total response is the same as that displayed in Fig. 4 and is equal to the sum of the other two responses. The calibration marker on the right of Fig. 5 applies to all the responses in that row. Notice that the gain is 5-times higher for the records in the top row.

The vertical dashed line in Fig. 5 marks the mean implicit time (27.7 msec) for the summed peripheral responses from the control subjects. Six of the patients show a delayed peripheral response relative to the controls and these delays are of comparable magnitude to those measured with full-field flicker; a seventh patient (P3) does not have a detectable peripheral response. As mentioned above (Fig. 4), four of the patients (P3, P8, P7, P1) have Total responses within the range of normal implicit times. On the other hand, only P1, the patient with the normal full-field flicker implicit time, has a peripheral response with a normal implicit time. Thus,

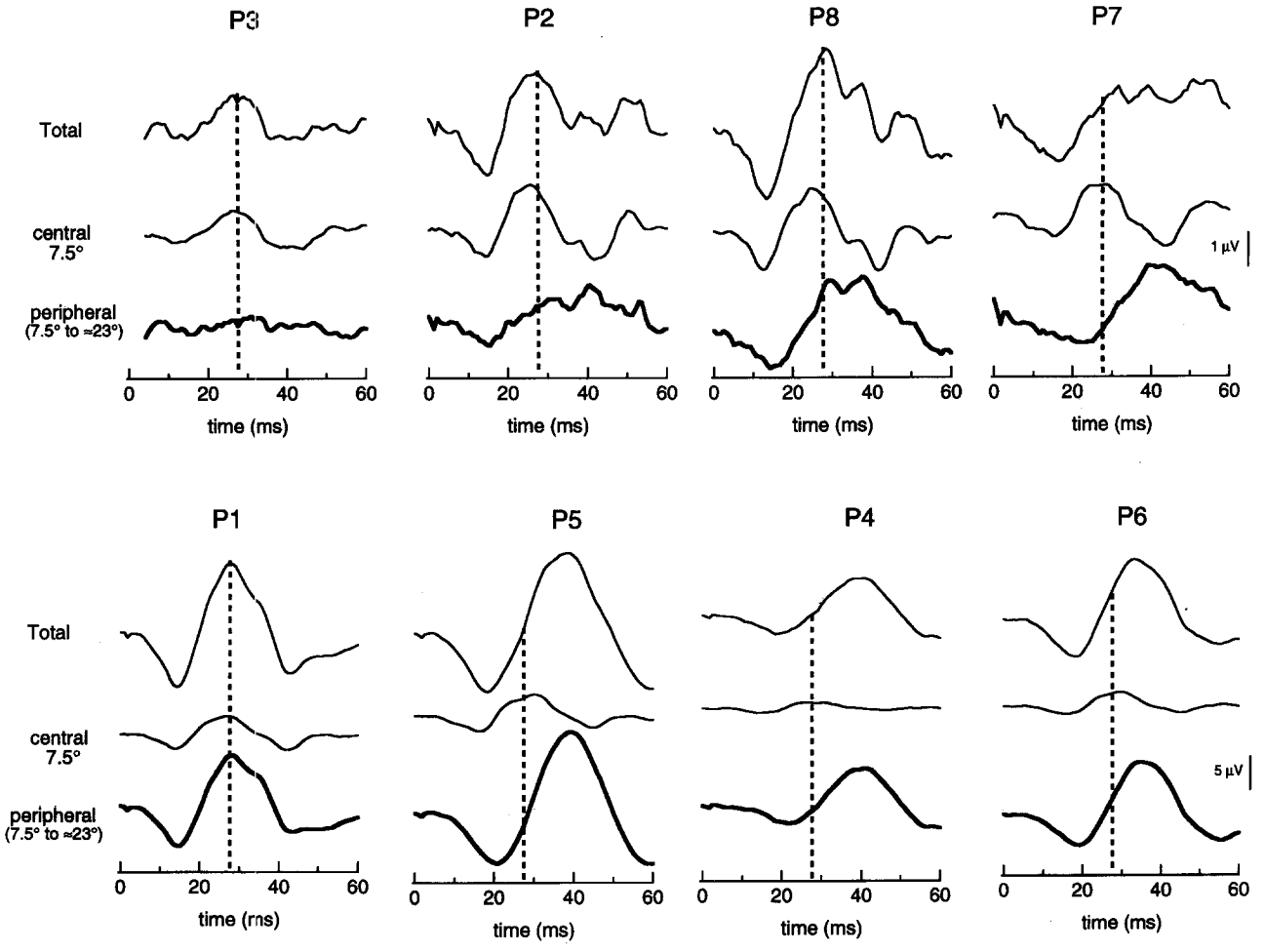

FIGURE 5. In each panel, the summed multi-focal ERGs for all 103 responses (Total—first row), for the central 19 responses (central 7.5 deg—second row), and for the remaining 84 responses (peripheral—third row) are shown for each of the eight patients with RP. The vertical dashed line indicates the mean implicit time (27.7 msec) for the peripheral responses from the controls.

there is general agreement between the summed peripheral multi-focal response and the full-field flicker responses. The peripheral responses in Fig. 5 are probably more representative of the total response one would obtain with the larger, full-field (Ganzfeld) display which, of course, includes much more of the periphery. In the case of P3 who does not show a response to the peripheral hexagons, the full-field flicker ERG which is small in amplitude (see Table 1) is presumably due to responses from peripheral regions beyond 23 deg.

Analysis of individual multi-focal responses. The amplitudes and implicit times of the individual responses for all subjects were measured as described in the Methods section. For each location, the values for the four control subjects were averaged. As expected from previous multi-focal ERG studies, there was relatively little variation (SD = 0.85) in implicit times across the normal retina (e.g. Sutter & Tran, 1992; Parks, Keating, Williamson, Evans, Elliot, & Jay, 1996; Verdon & Haegerstron-Portnoy, 1996). The central responses tended to be slightly longer (see above). However, the longest mean implicit time was associated with the hexagon falling closest to the blind spot*; here the implicit time was 3.7 msec longer than the grand average.

*Although the blind spot shows up as a slightly smaller and slower response in the data for the four normal controls, it is not very obvious in the records of some subjects. For example, the blind spot is difficult to locate in the records of Fig. 1(B). And, it is never as obvious in multi-focal studies (e.g. Sutter & Tran, 1992; Bearse & Sutter, 1996; Kondo *et al.*, 1995; Parks *et al.*, 1996) as it is in the behaviorally measured visual fields (see Figs 12 and 13). Keeping in mind that the multi-stimulation technique measures the response associated with each hexagon, there are two possible explanations. First, to see a clear blind spot a hexagon must fall within the blind spot and fixation must be good enough to keep it there during the recording. The hexagons in this study are relatively large and it is unlikely that any given hexagon will be positioned completely within the blind spot. It is possible to show a clearer blind spot with smaller hexagons (Sutter & Tran, 1992; Bearse & Sutter, 1996), but even with smaller hexagons there are measurable responses in the region of the blind spot. This raises the possibility of a second contributing factor, stray light. The retinal area stimulated must be larger than the retinal image of the hexagon. It is difficult to know how much to attribute to each of these factors. However, it is important to emphasize that the responses here are reasonably local. The fact that approximately equal size responses are produced when the hexagons are scaled to approximate the cone density [see Fig. 1(B)] argues for the local nature of these responses (e.g. Sutter & Tran, 1992). Furthermore, the agreement here with the patients' fields (see P1, for example) also supports a reasonably localized response.

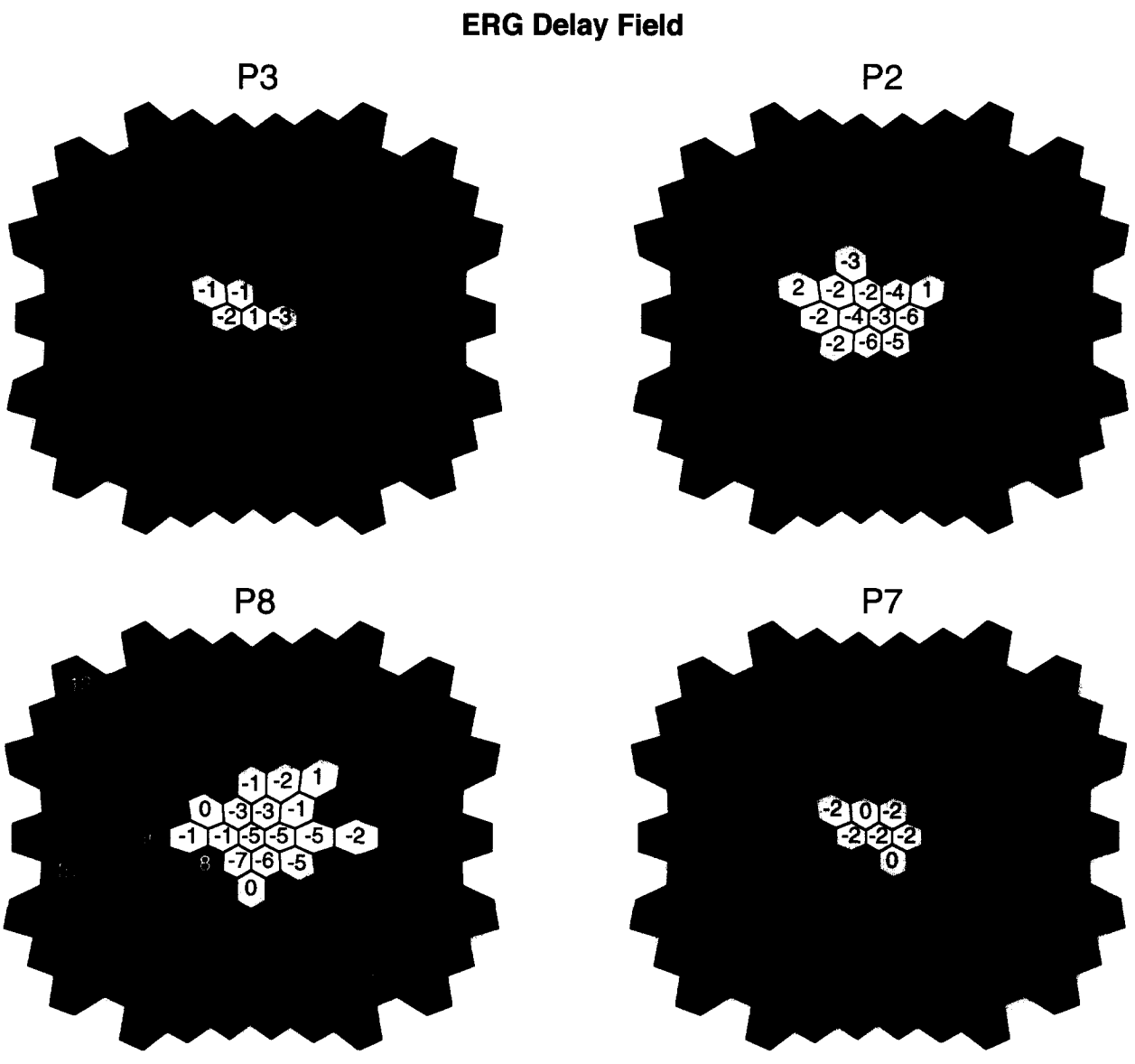

FIGURE 6. ERG delay fields calculated by subtracting the mean implicit time for the controls from the implicit time for the patient's response at each location. The numbers in these fields are the delays rounded to the nearest millisecond for presentation. The black hexagons indicate that the response amplitude did not meet the criterion value of 90 nV (see Methods). The clear regions signify that the delay was less than 1.7 msec (< + 2 SD); the light gray regions signify that the delay was between 1.7 and 3.4 msec (+2 to +4 SD); and the darkest gray regions signify that the delay was greater than 3.4 msec (> + 4 SD). [Note that since the numbers in these figures have been rounded, the same delay, for example 2 msec, can appear as clear or as light gray depending upon whether it was less than or greater than 1.7 msec.]

To take into consideration possible variations across the field, a delay was calculated for each of the patients' responses by comparing it with the normal value at the same location. Specifically, the delay for a particular response was equal to its implicit time minus the mean normal implicit time for that point.

Figures 6 and 7 show the "delay fields" for all eight patients. The numbers in these fields are the delays rounded to the nearest millisecond for presentation. The black hexagons indicate that the response amplitude did not meet the criterion value of 90 nV (see Methods). The clear regions signify that the delay was less than 1.7 msec; the light gray regions signify that the delay was between 1.7 and 3.4 msec; and the darkest gray regions signify that the delay was greater than 3.4 msec. For comparson, Fig. 1(C) shows the delay field for one of the control subjects [same subject as in Fig. 1(B)]. To obtain an estimate of normal variation in delays, the standard deviation of the 412 delays (four subjects and 103 responses) for the control subjects' responses was calculated. This value of 0.85 msec was used in setting the cut-offs for the shading in Figs 6 and 7. That is, the clear, light gray, and dark gray regions indicate delays within +2 SD (< 1.7 msec), +2 to +4 SD (1.7 to 3.4 msec), and greater than +4 SD (>3.4 msec), respectively. [Note that since the numbers in these figures have been rounded the same delay, for example 2 msec, can appear as clear or as light gray depending upon whether it was less than or greater than 1.7 msec.]

All patients show at least some central responses that

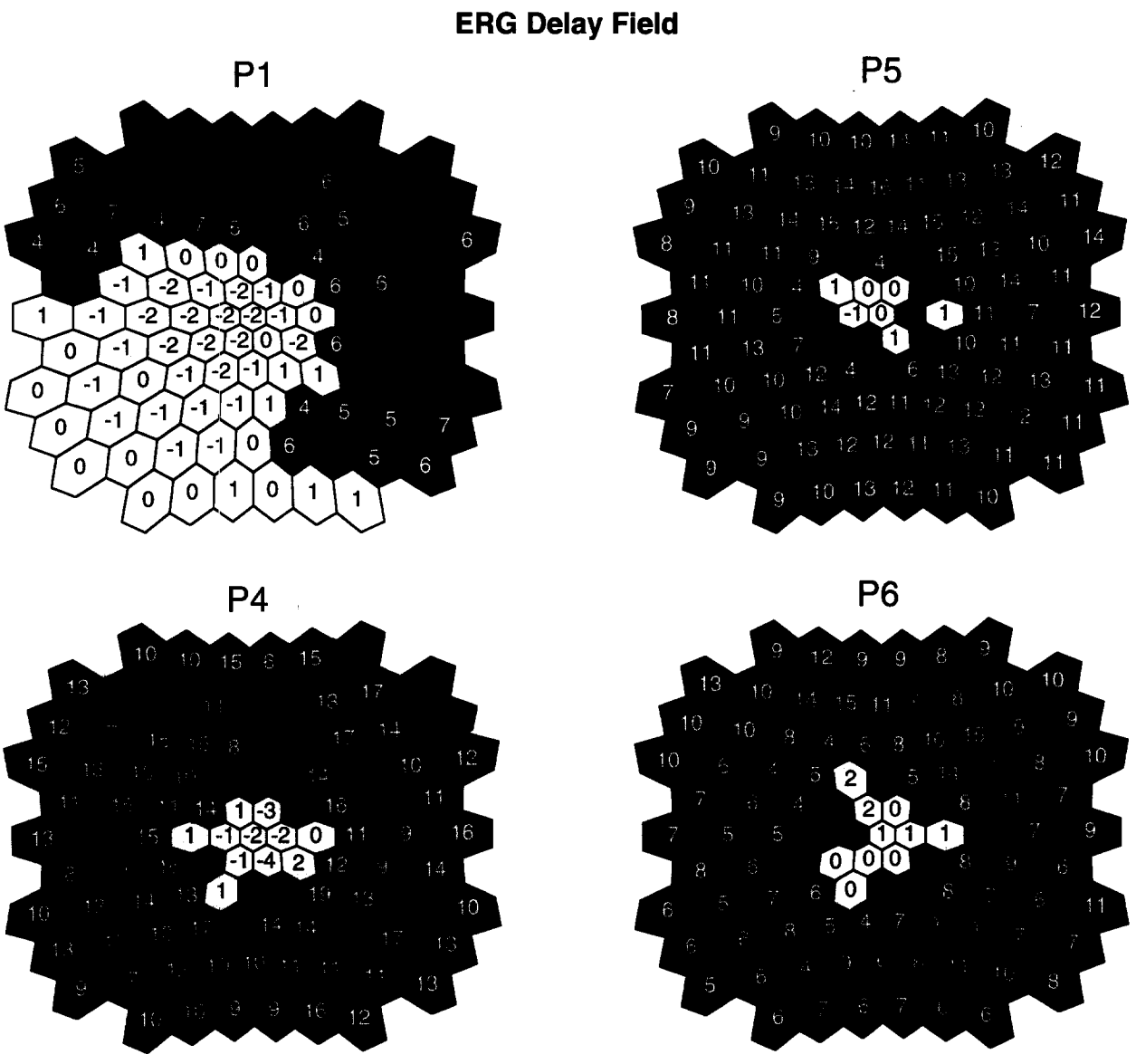

FIGURE 7. Same as for Fig. 6.

are not delayed. On the other hand, with the exception of patient P1, few responses outside the central 7.5 deg (central 19 responses) show normal timing. Recall that P1 was the only patient with a normal implicit time for the full-field 30 Hz response. Notice how abruptly these delay fields change for patients P5, P4, and P6 in Fig. 7. Regions with delays of more than 10 msec are adjacent to regions with near normal timing.

Figures 8 and 9 show a similar analysis for the peak-to-trough amplitudes obtained as described in the Methods. The numbers in these fields are the difference at each point between the patient's amplitude and the mean normal amplitude for that point. These differences in trough-to-peak amplitude were rounded and expressed in units of 100 nV for clarity of presentation. The clear regions in these "loss" fields signify a decrease in amplitude of less than 162 nV (< -2 SD); the light gray regions signify a decrease between 162 and 324 nV (-2 to -4 SD); and the darkest gray regions signify a decrease greater than 324 nV (> -4 SD). Figure 1(D) shows the amplitude loss field for one of the control subjects [same subject as in Fig. 1(B)]. In contrast to the delay fields which had normal central timing, only two of the patients (P1 and P5) show central responses within 2 SD of the normal amplitude. In fact, these are the only patients with near normal amplitudes anywhere in their fields.

Figure 10(A) is a correlation plot in which timing delays and amplitude losses are plotted for individual responses from the four control subjects. Delay and amplitude loss are not correlated in the control subjects. Notice that the delays in implicit time fall in a very narrow range with a SD of 0.85 msec. The solid lines in all panels mark the boundaries for a delay (horizontal line) or amplitude loss (vertical line) that exceeds 2 SD. The values for the patients' records in Fig. 2 are shown in Fig. 10(B) and those for the records in Fig. 3 are in Fig. 10(C, D). The pattern of results is quite similar for the records of the six patients in (B) and (C). All points cluster to the left of the vertical line marking the limits of

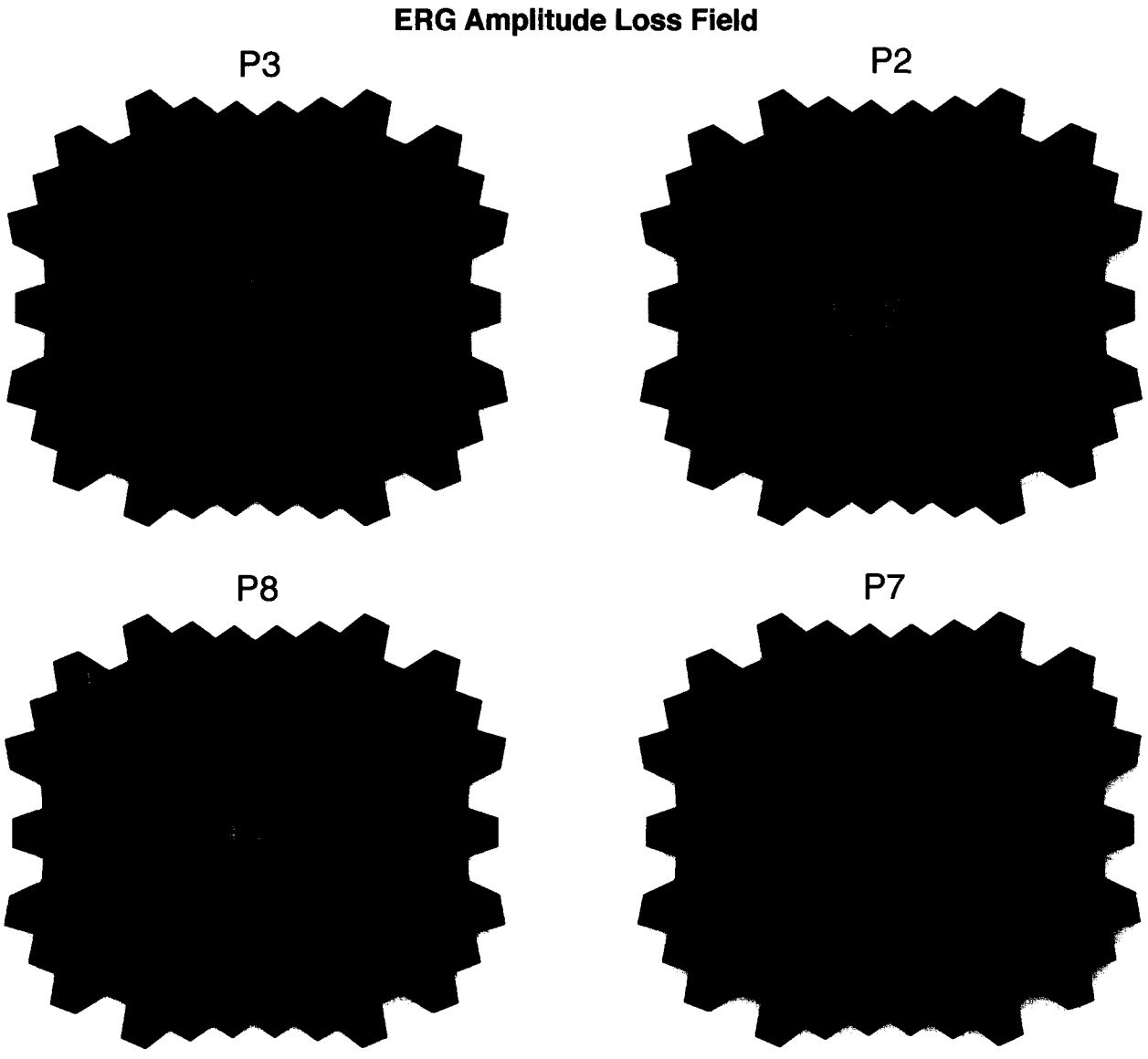

FIGURE 8. ERG Amplitude loss fields calculated by subtracting the mean trough-to-peak amplitude for the controls from the trough-to-peak amplitude for the patient's response at each location. These differences in trough-to-peak amplitude were rounded and expressed in units of 100 nV for clarity of presentation. The clear regions indicate a decrease in amplitude of less than 162 nV (< -2 SD); the light gray regions signify a decrease between 162 and 324 nV (-2 to -4 SD); and the darkest gray regions signify a decrease greater than 324 nV (> -4 SD).

normal amplitude (i.e., all are significantly decreased in amplitude), but the points show a range of delays from normal to markedly delayed. There are significant negative correlations between loss in amplitude and delay in timing for these patients, although the correlation coefficients tend to be relatively low ($r = 0.2$ to 0.5). The data for the other two patients (panel D) also show negative correlations between delays and amplitude losses. However, they show different patterns than do the other six patients. Most notably, P5 has the only responses that are near normal in amplitude but markedly delayed (see arrow). In addition, P1 has no timing delays greater than 7 msec, no matter how reduced the amplitude (see arrow).

Control experiment: the effects of mean luminance

A number of studies have shown that the delays in the full-field ERG in patients with RP cannot be mimicked by a simple change in sensitivity to light (e.g. Berson *et al.*, 1969; Massof *et al.*, 1986; Seiple *et al.*, 1986; Miller & Sandberg, 1991; Seiple, Holopigian, Greenstein, & Hood, 1993; Hood & Birch, 1996). To examine the effects of a change in sensitivity that acted to decrease all lights by the same factor, multi-focal ERGs were obtained for a range of mean luminance levels. Figure 11 shows the summed total multi-focal ERGs from a control subject for a mean luminance ranging from 200 cd/m^2 (the level used in this study) to 10 cd/m^2. In all cases, the contrast was as close to 100% as the monitor allowed. The amplitude decreased monotonically with decreased mean luminance, but the implicit time remained approximately the same until the luminance was decreased by more than a factor of 10. For the 10 cd/m^2 condition, the implicit time was delayed by 7.8 msec. Thus, we cannot rule out a

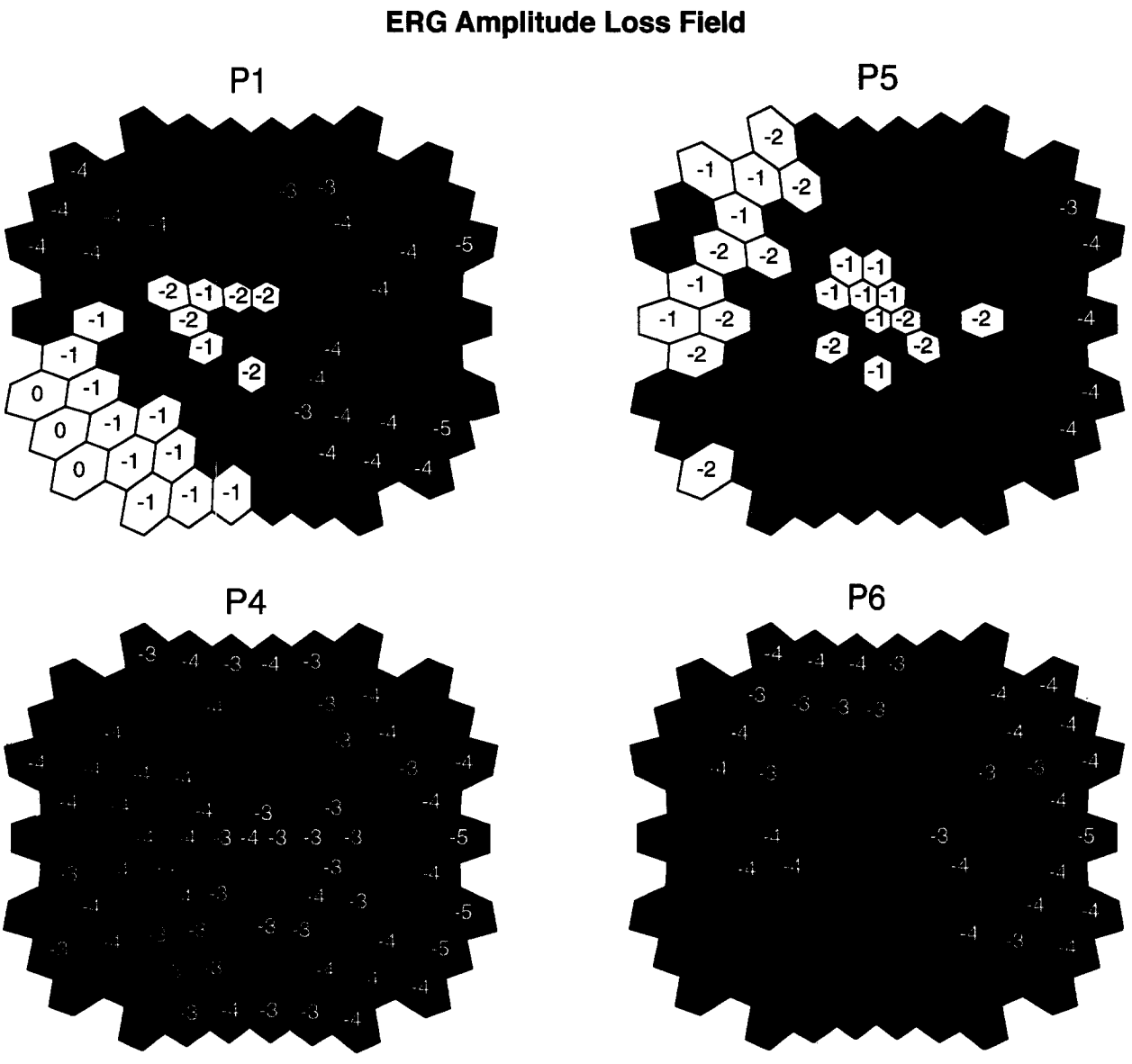

FIGURE 9. Same as for Fig. 8.

simple change in sensitivity as the cause of the delays seen in the patients with very small responses (e.g. P2, P8, P7). However, local sensitivities would have to be depressed by a factor of more than 20 to account for the delays. On the other hand, a simple decrease in sensitivity cannot account for the relatively large, but delayed responses seen in other patients (e.g. P4, P5, P6).

Discussion: Experiment 1

It has been known for over 25 years that full-field, cone ERGs are delayed in patients with RP and extensive work has been done to establish the causes and sites of these delays. However, relatively little is known about how these delays relate to local retinal function. There are previous reports that the central focal ERG can be normal in patients with delayed, full-field ERGs (Sandberg *et al.*, 1978, 1979; Seiple *et al.*, 1986). One of these studies also obtained a delayed focal ERG to a relatively large peripheral stimulus in three patients with dominant RP (Sandberg *et al.*, 1979). Further, there is a report of a patient with RP whose summed multi-focal responses showed normal central implicit times but delayed peripheral timing.‡ Based upon these findings, it is not surprising that our group of patients with reasonably good central vision has central responses with normal timing but peripheral responses that are delayed. The present results show, however, that individual patients can have a wide range of delays within their retina. In fact, a range of multi-focal implicit times from normal to markedly delayed was observed in patients with full-field, 30 Hz ERGs of normal (e.g. P1) and delayed (e.g. P4, P5, P6) timing.

It is clear from the results of the current study why delayed cone ERGs are so common in patients with RP. The summed peripheral responses (outside 7.5 deg) are delayed in patients with delayed full-field 30 Hz

‡The summed multi-focal responses for this patients are in Fig. 8 of an article in Japanese by Kondo *et al.* (1996).

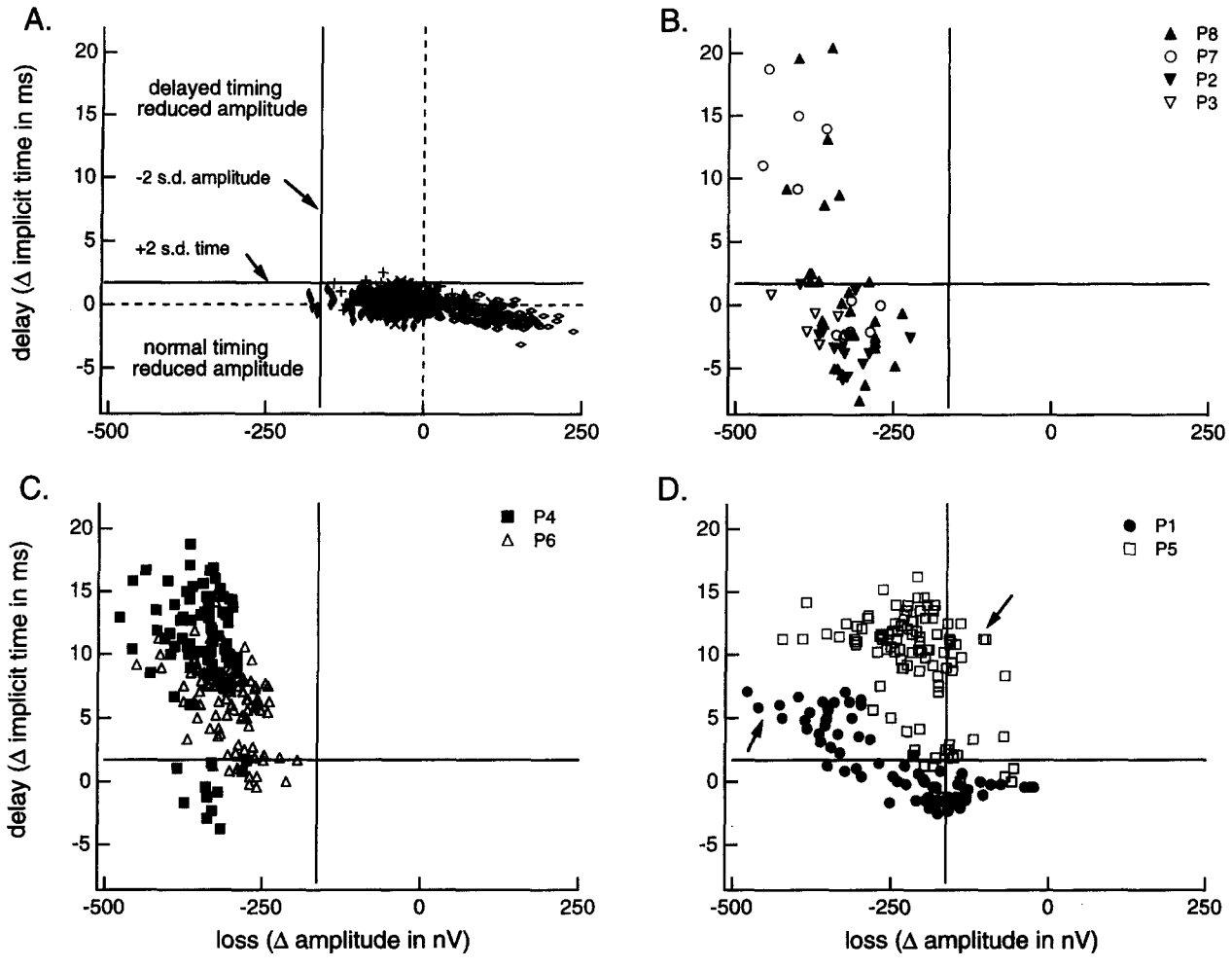

FIGURE 10. (A) The implicit time delay relative to the mean of the controls is plotted against the loss in amplitude for each response from each of the four control subjects. The data for each of the four controls are shown as a different symbol. (B), (C), and (D) show the same plots for the eight patients. See text for details.

responses (Fig. 5). In fact, nearly *all* the peripheral responses in the patients with delayed 30 Hz responses are delayed, even in the case (P5) where the amplitude is near normal (see Figs 6 and 7). It is also clear why these patients have full-field responses that are reduced in amplitude. Only P1 and P5 had responses of near normal amplitude anywhere in their fields.

The present results also help explain the variety of waveforms seen in full-field, cone ERGs from patients with RP. Full-field ERGs with reduced amplitudes can have normal timing, be markedly delayed, or just look very strange (e.g. see Fig. 3 in Berson *et al.*, 1969; see Fig. 1 in Hood & Birch, 1995). The results in Fig. 5 make clear how the full-field waveform will depend upon the timing and extent of the functional retina. To a first approximation the summed Total ERGs (see Fig. 5) from all patients but P1 can be seen as the sum of a central response with normal timing and a markedly delayed peripheral response. The waveform of the Total, and presumably the full-field ERG, depends upon the relative size of the peripheral response. If the peripheral response is relatively large, then the Total response is delayed (e.g. P5, P4, P6). If the peripheral reponse is near zero, then the Total response is very small with normal timing (e.g. P3 and P2). And, if central and peripheral responses have nearly equal amplitudes, the Total response can have an unusual looking waveform (e.g. P7).

While we have focused thus far on the similarities among the patients' results, there are clear differences that require explanation. The records in Fig. 2 are from patients who appear to have little or no peripheral function remaining. But, how are we to interpret the results from the patients in Fig. 3? Here we find a range of amplitudes and timing. In fact, among these patients can be found peripheral responses with near normal amplitude and timing (e.g. P1), near normal amplitude and abnormal timing (P5), abnormal amplitude but normal timing (e.g. P1), as well as abnormal amplitude and very delayed timing (P4, P5, P6). Some, but not all, of this variation can be understood based upon the patients' visual fields studied in Experiment 2.

EXPERIMENT 2

Cone visual fields of patients with RP show a range of sensitivity losses. Because of the difficulty in obtaining focal ERGs with conventional methods, there is little in the literature that allows a comparison of cone visual

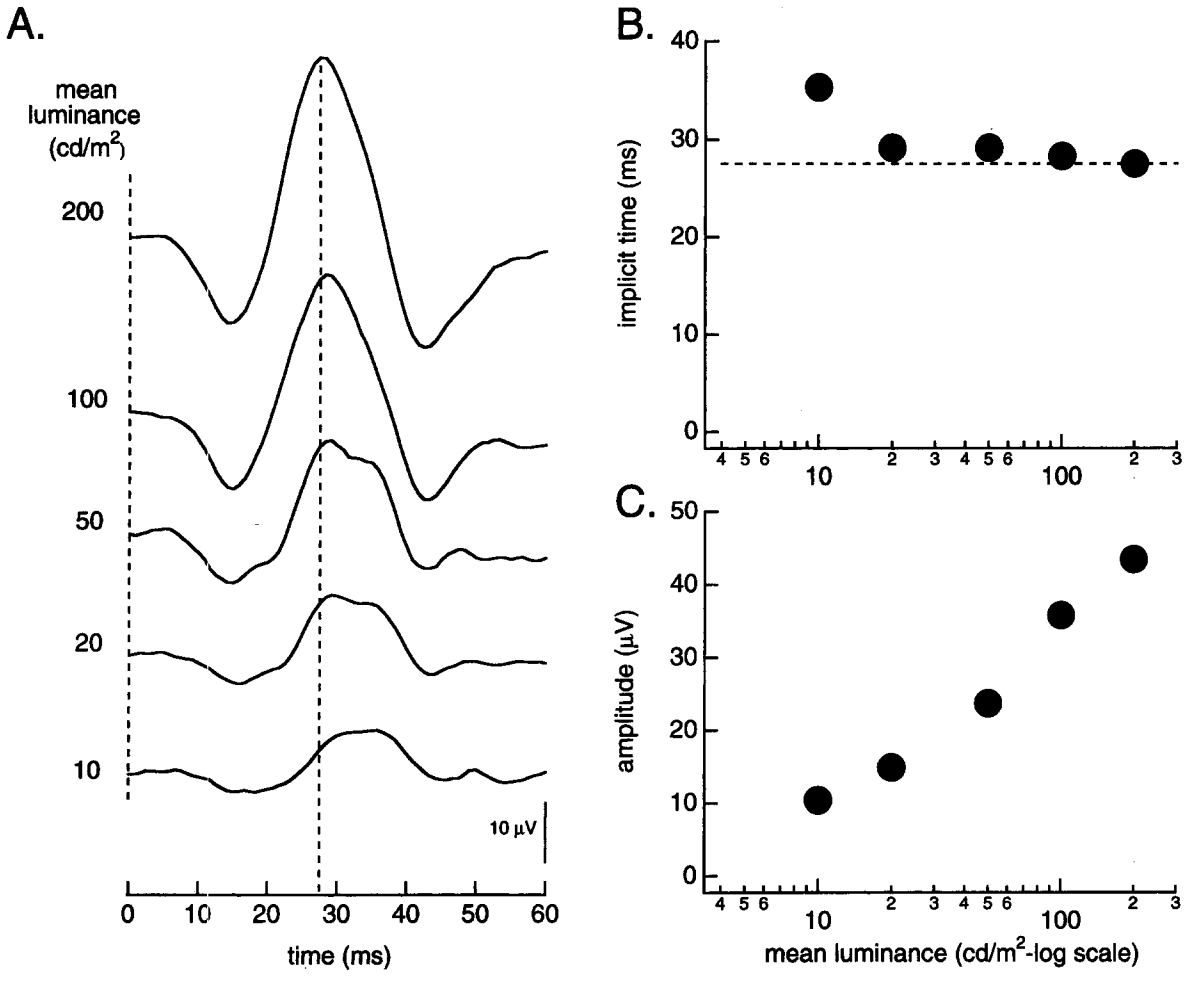

FIGURE 11. (A) Summed multi-focal responses for all 103 responses to displays with different mean luminances. (B) The implicit times for the records in (A). (C) The trough-to-peak amplitudes for the records in (A).

fields with local retinal activity outside the central retina. It appears from the results of Experiment 1 that the multi-focal technique offers a way to assess local retinal function in patients with RP. Here we measure visual sensitivity in the same locations at which multi-focal ERGs were obtained as part of Experiment 1.

Methods

All patients had visual fields measured on a Humphrey perimeter using the 30-2 central threshold program. This program measures 76 thresholds with a 40′ test light presented on a grid of points separated by 6 deg. When it was clear that the quality of the multi-focal data would allow for quantitative comparisons with field data, a custom display was designed for the Humphrey which more closely matched the multi-focal display. In particular, thresholds were measured at 102 locations with the 40′ test light centered in the middle of each hexagonal area in the multi-focal display. (The Humphrey program does not allow a central point to be measured as part of the custom display). The background luminance was 10 cd/m^2.

We were able to re-test the patients within several months of the multi-focal test. The same four subjects as in Experiment 1 served as the controls. The visual losses calculated for the patients based upon this small control group closely resembled those obtained with the standard Humphrey 30-2 program and the Humphrey age-matched norms. Only the results for the custom display are presented below.

Results

Figures 12 and 13 show the Humphrey visual fields. The visual fields are expressed in terms of the log of the ratio of the patient's threshold to the mean threshold of the control group (see Methods). For example, a value of 0 corresponds to 0 log unit or a threshold intensity that was equal to the value for the control group. In addition, a value of 0.3 corresponds to a threshold that is 0.3 log unit above the mean of the control group or a threshold that is twice that value. The three points identified as NaN (for "not-a-number") are the central point and the two points falling on the blind spot of one or more of the control subjects. To obtain an estimate of normal variation in the loss in log threshold for the customized field, the standard deviation of the 400 measures (four subjects and 100 measurements) for the control subjects' responses was calculated. The clear regions signify that the patient's threshold within 0.5 log unit ($< +2$ SD) of the mean of the four control subjects; the light gray regions signify

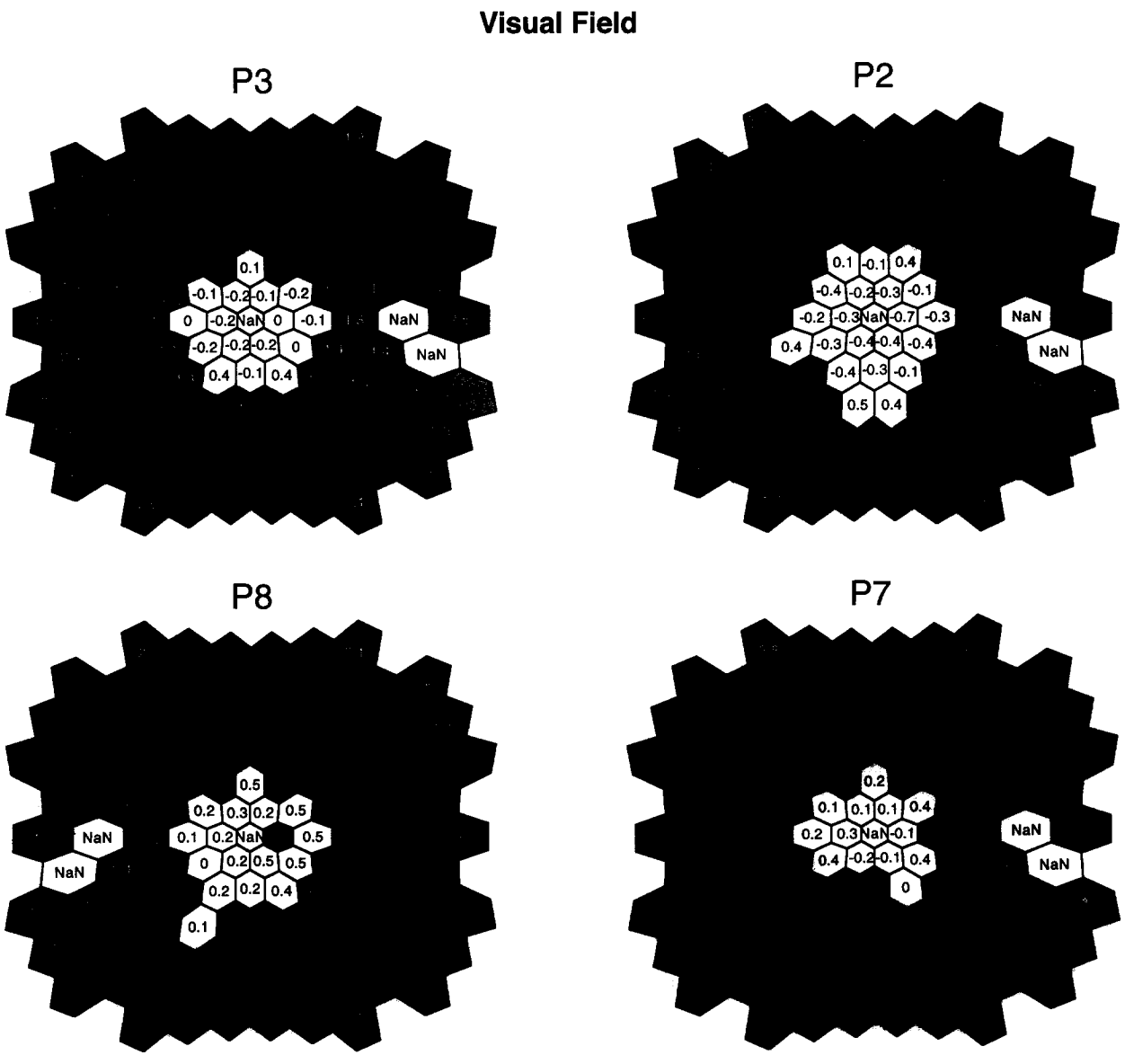

FIGURE 12. The visual fields for the modified Humphrey threshold program. The number at each point is the log of the ratio of the patient's threshold to the mean threshold of the control group for that point. The clear regions signify that the patient's threshold at that point was within 0.5 log unit (< + 2 SD) of the mean, the light gray regions signify values between 0.5 and 1.0 log unit (+2 to +4 SD), and the dark gray signify values greater than 1.0 log unit (>4 SD). The three points identified as NaN are the central point and two points falling on the blind spot of one or more of the control subjects.

values between 0.5 and 1.0 log unit (+2 to +4 SD); and the dark gray regions signify values greater than 1.0 log unit (>4 SD).

Notice that all patients show regions of near normal sensitivity in the central 5 deg (the central seven hexagons). These are regions which showed reduced amplitude but normal timing in all patients' multi-focal ERGs (see Fig. 4). In the peripheral regions, there is a range of sensitivity losses both within and across patients.

There is general agreement between the multi-focal ERGs and the visual fields. For example, the patients with the poorest peripheral responses (Fig. 2) show depressed sensitivity outside the central 7.5 deg or so (central 19 hexagons) (Fig. 12). And, the patient with the best peripheral responses and the normal 30 Hz flicker (P1 in Fig. 3) has a lower quadrant with near normal sensitivity (Fig. 13). Although there is qualitative agreement between the multi-focal and behavioral measures, there are two clear differences. First, the central area of the visual field with near normal sensitivity appears larger than the areas with ERG normal timing in most of the patients. This is most obvious for P5 and P6 but, with the exception of P1 and possibly P8, holds for the other patients as well. Second, although losses in sensitivity of 1 log unit or more are associated with small, delayed multi-focal ERGs, P1 shows relatively small delays in regions of loss greater than 1 log unit and P5 shows relatively large responses in such regions.

The points of agreement and disagreement between the ERG and behavioral measures can be seen quantitatively in Figs 14 and 15, where the change in implicit time (Fig. 14) and amplitude (Fig. 15) are plotted against the change in log threshold. In each figure, the data for the control subjects are in the left panel and those for the patients are

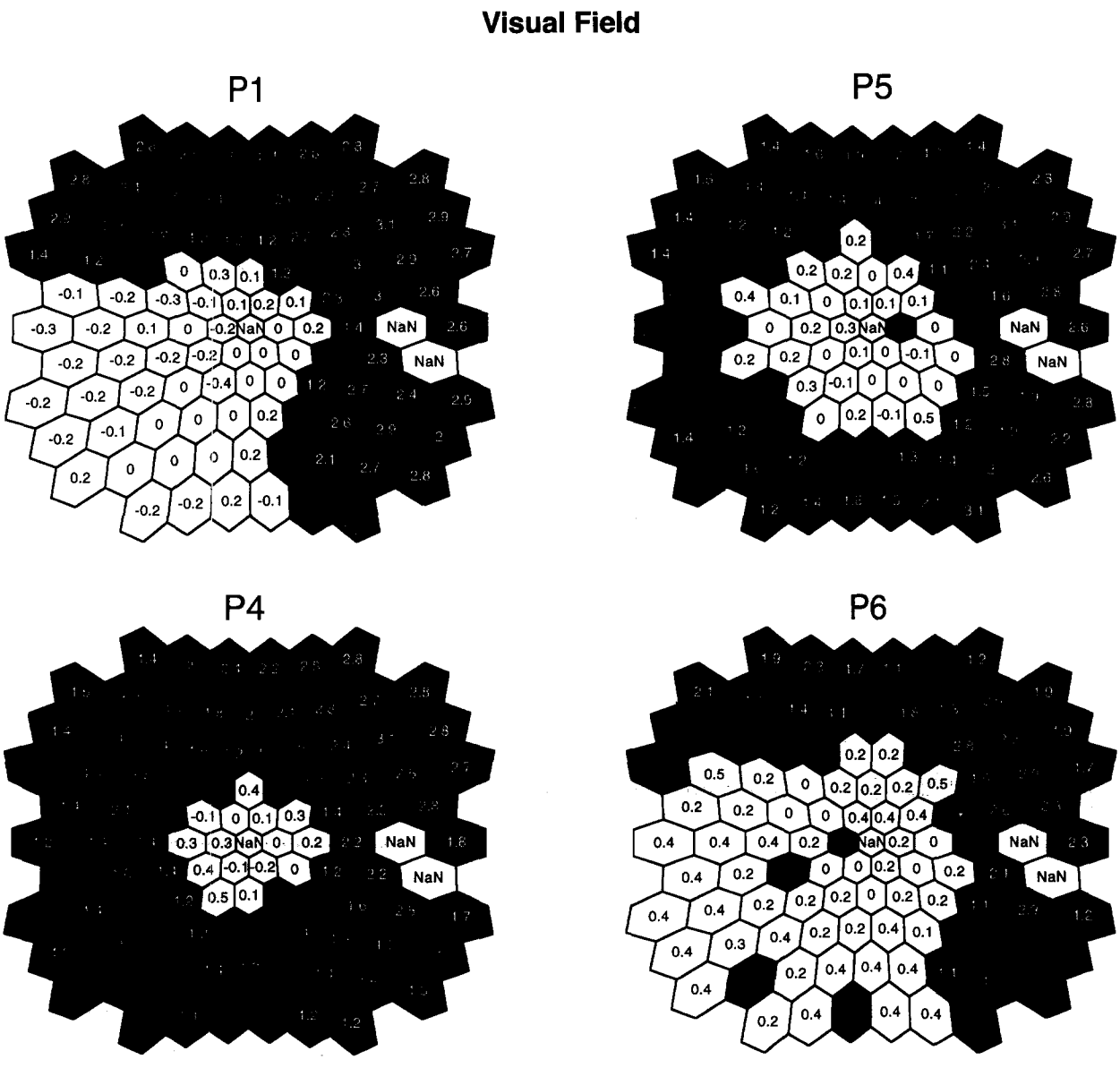

FIGURE 13. Same as Fig. 12 for the other four patients.

in the right panel. The points with significantly reduced sensitivity fall to the right of the vertical line in Fig. 14. Notice that most of these points are in the upper right quadrant, the region of delayed timing. Thus, in general, areas with reduced sensitivity (elevated log thresholds) have delayed ERGs and areas with ERGs with near normal timing have near normal sensitivity. The reverse is not strictly true. In particular, some areas that are delayed have near normal sensitivity [upper left quadrant in Fig. 14(B)]. The data from P5 and P6, in particular, show regions with near normal sensitivity but delayed responses (Figs 7 and 13).

Unlike delays in implicit time, amplitude loss does not appear to be a particularly good predictor of which region has normal sensitivity, as indicated in Fig. 15. In this figure, the loss in amplitude is shown vs the loss in log sensitivity; the multi-focal ERGs that did not meet the criterion amplitude are plotted arbitrarily at −600 nV. As mentioned above, the patients show few responses within the normal range of amplitudes. Reduced amplitude does not necessarily mean abnormal sensitivity, nor does normal amplitude necessarily indicate normal sensitivity. In fact, some of the points with normal sensitivity had multi-focal responses that did not meet the criterion amplitude.

Discussion: Experiment 2

The multi-focal technique, as used here, allows a comparison between local retinal activity and visual sensitivity. There have been a few reports of general agreement between visual field losses and reduced multi-focal ERGs in patients with RP (e.g. Kondo *et al.*, 1995; Seeliger *et al.*, 1996; Hood *et al.*, 1996), but little has been done in the way of quantitative comparisons. In the present study, the amplitudes and implicit times of the individual multi-focal responses were compared with local visual field losses.

Although the amplitude of the multi-focal response shows a qualitative agreement with the visual field, we find that the correlation between sensitivity and ampli-

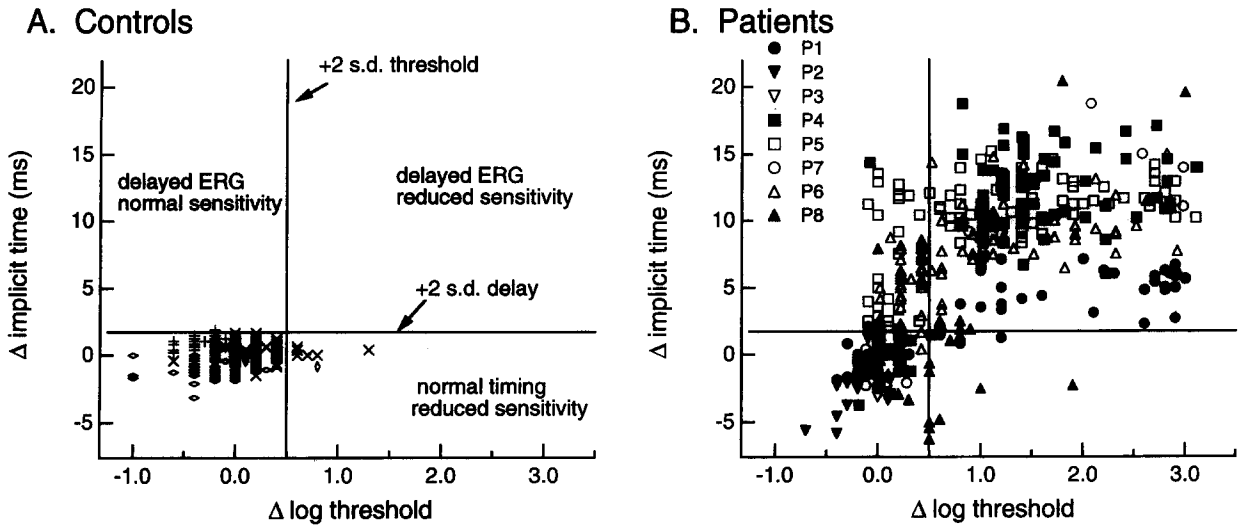

FIGURE 14. (A) The implicit time delay relative to the mean of the controls is plotted against the elevation in log threshold, relative to the mean of the controls for each location for the four control subjects. (B) Same as in (A) but for the patients for whom modified Humphrey fields were available. See text for details.

tude is poor. Reduced amplitudes are seen in many regions with good sensitivity and timing, and, a near normal amplitude does not assure near normal sensitivity (see P5 in Fig. 15). This suggests that correlations between full-field cone ERG amplitudes and sensitivity losses will always be imperfect in patients with RP.

While the amplitude of the multi-focal response is not a good predictor of behavioral sensitivity, there is a clear relationship between implicit time and behavioral sensitivity. A normal implicit time is associated with near normal sensitivity. In particular, if the local retinal area has lost 0.5 log unit or more in sensitivity, then the multi-focal ERG is usually delayed and this delay is often about the same as it is for areas with far greater losses in sensitivity. Delays in the multi-focal responses seem to be an early indication of local retinal damage in patients with RP. As further evidence that delayed responses may be a very sensitive measure of local retinal damage, notice that delayed responses are found in regions outside the central 5 deg with near normal sensitivity in three patients (P4, P5, P6).

The present results also raise some questions about the nature of the retinal damage responsible for sensitivity loss in patients with RP. Consider, for example, the responses from areas depressed by 2 or more log units. Patient 1 shows multi-focal ERGs that are very small and moderately delayed while the multi-focal ERGs from patient 5 are relatively large and very delayed. It appears as if the damage to the cone system in these two patients is different.

CONCLUSION

The multi-focal ERGs and visual fields from the

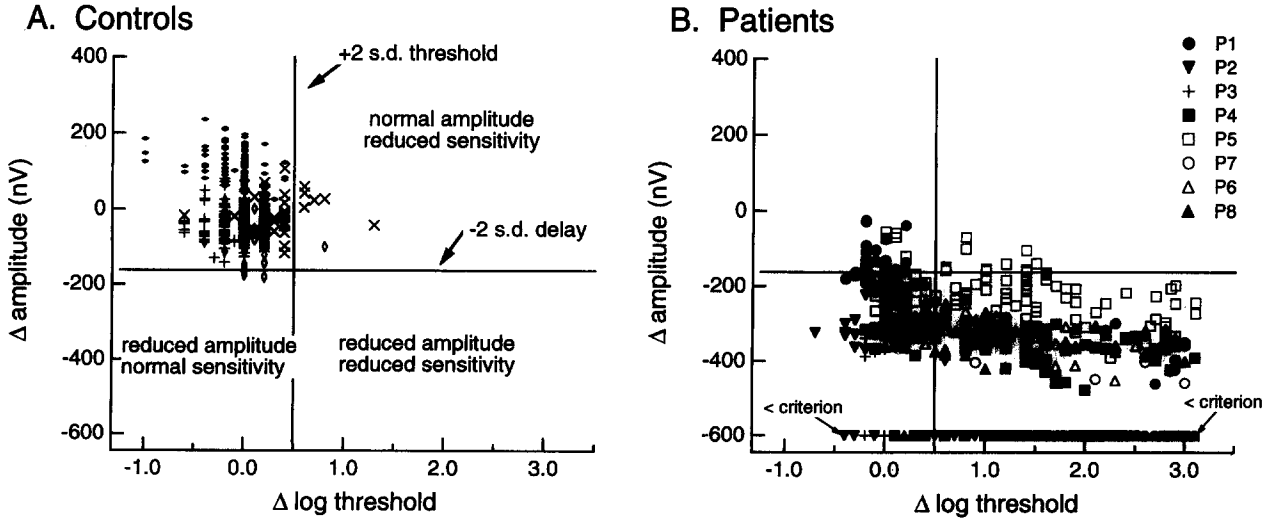

FIGURE 15. (A) The loss in amplitude relative to the mean of the controls is plotted against the elevation in log threshold relative to the mean of the controls for each location for the four control subjects. (B) Same as in (A) but for the patients for whom modified Humphrey fields were available. See text for details.

patients clarify the bases of some well established findings. For example, they provide a local retinal basis for the delays seen in the full-field ERG recorded from most, but not all, patients and for the variety of waveforms of full-field ERGs recorded from different patients. They also provide an explanation for the difficulty in obtaining good quantitative agreement between full-field ERG and visual field measures in some patients. For example, they provide a basis for the finding that full-field ERGs are often smaller than one would predict based upon visual field data. On the other hand, the results raise new questions about the mechanisms responsible for the changes in the ERG in patients with RP. Areas with extreme sensitivity loss show multi-focal responses with a wide range of amplitudes and implicit times across patients suggesting different mechanisms of disease action in different patients. Finally, we conclude that the multi-focal ERG should prove useful, both in studies designed to test hypotheses about the action of RP and in trials to assess a particular treatment. The delay fields look like a particularly sensitive measure of local retinal health.

REFERENCES

Arden, G. B., Carter, R. M., Hogg, C. R., Powell, D. J., Ernst, W. J. K., Clover, G. M., Lyness, A. L. & Quinlan, M. P. (1983). Rod and cone activity in patients with dominantly inherited retinitis pigmentosa: comparisons between psychophysical and electroretinographic measurements. *British Journal of Ophthalmology, 67*, 405–418.

Bearse, M. A. & Sutter, E. E. (1996). Imaging localized retinal dysfunction with the multifocal electroretinogram. *Journal of the Optical Society of America, 13*, 634–640.

Berson, E. (1993). Retinitis pigmentosa: the Friedenwald lecture. *Investigative Ophthalmology and Visual Science, 34*, 1659–1676.

Berson, E., Gouras, P. & Hoff, M. (1969a). Temporal aspects of the electroretinogram. *Archives of Ophthalmology, 81*, 207–214.

Berson, E., Gouras, P., Gunkel, R. D. & Myrianthopoulos, N. C. (1969b). Rod and cone responses in sex-linked retinitis pigmentosa. *Archives of Ophthalmology, 81*, 215–225.

Berson, E. L. & Kanters, L. (1970). Cone and rod responses in a family with recessively inherited retinitis pigmentosa. *Archives of Ophthalmology, 84*, 288–297.

Biersdorf, W. R. (1982). Temporal factors in the foveal ERG. *Current Eye Research, 1*, 717–722.

Hood, D. C. & Birch, D. G. (1995). Abnormal cone receptor activity in patients with hereditary degeneration. In R. E. Anderson *et al.*, eds, *Degenerative diseases of the retina* (pp. 349–358).

Hood, D. C. & Birch, D. G. (1996). Abnormalities of the retinal cone system in retinitis pigmentosa. *Vision Research, 36*, 1699–1709.

Hood, D. C., Holopigian, K., Greenstein, V. C., Seiple, W., Sutter, E. E. & Carr, R. E. (1996). Do the delays in the cone ERG from patients with RP indicate global retinal damage? *Investigative Ophthalmology and Visual Science, 37 (Suppl.)*, S341.

Hood, D. C., Seiplee, W., Holopigian, K. & Greenstein, V. C. (1997). A comparison of the components of the multi-focal and full-field ERGs. *Visual Neuroscience, 14*, 533–544.

Keating, D., Parks, S., Williamson, T. H., Evans, A. L., Jay, J. L. & Elliott, A. T. (1996). The effect of pupil dilation, retinal blur and filter bandwidth on the multi-focal ERG. *Investigative Ophthalmology and Visual Science, 37 suppl*, S346.

Kondo, M., Miyake, Y., Horiguchi, M., Suzuki, S. & Tanikawa, A. (1995). Clinical evaluation of multifocal electroretinogram. *Investigative Ophthalmology and Visual Science, 36*, 2146–2150.

Kondo, M., Miyake, Y., Horiguchi, M., Suzuki, S., Ito, Y. & Tanikawa, A. (1996). Normal values of retinal response densities in multi-focal electroretinogram. *Journal of Japanese Ophthalmological Society, 100*, 810–816.

Massof, R., Johnson, M., Sunness, J., Perry, C. & Finkelstein, D. (1986). Flicker electroretinogram in retinitis pigmentosa. *Documenta Ophthalmologica, 62*, 231–245.

Massof, R. W., Wu, L., Finkelstein, D., Perry, D., Starr, S. J. & Johnson, M. A. (1984). Properties of electroretinographic intensity-response functions in retinitis pigmentosa. *Documenta Ophthalmologica, 57*, 279–296.

Miller, S. & Sandberg, M. (1991). Cone electroretinographic change during light adaptation in retinitis pigmentosa. *Investigative Ophthalmology and Visual Science, 32*, 2536–2541.

Miyake, Y., Shiroyama, N., Ota, I. & Horiguchi, M. (1988). Oscillatory potentials in electroretinograms of the human macular region. *Investigative Ophthalmology and Visual Science, 29*, 1631–1635.

Nusinowitz, S. & Birch, D. G. (1997). Topography of rod and cone sensitivity loss in retinitis pigmentosa. In Lakshminarayanan, V. (Ed.) *Basic and clinical applications of vision science.* (pp. 237–240). Amsterdam: Kluwer.

Parks, S., Keating, D., Williamson, T. H., Evans, A. L., Elliot, A. T. & Jay, J. L. (1996). Functional imaging of the retina using the multifocal electroretinogram: a control study. *British Journal of Ophthalmology, 80*, 831–834.

Sandberg, M. A. & Ariel, M. (1977). A hand-held two channel stimulator ophthalmoscope. *Archives of Ophthalmology, 95*, 1881–1882.

Sandberg, M. A., Effron, M. H. & Berson, E. I. (1978). Focal electroretinograms in dominant retinitis pigmentosa with reduced penetrance. *Investigative Ophthalmology and Visual Science, 17*, 1096–1101.

Sandberg, M. A., Jacobson, S. G. & Berson, E. L. (1979). Foveal cone electroretinograms in retinitis pigmentosa and juvenile macular degeneration. *American Journal of Ophthalmology, 88*, 702–707.

Seeliger, M. W., Kretschmann, U. H., Ruther, R. W., Apfelstedt-Sylla, E. & Zrenner, E. (1996). ERG campimetry in retinitis pigmentosa. *Investigative Ophthalmology and Visual Science, 37 suppl*, S341.

Seiple, W., Holopigian, K., Greenstein, V. & Hood, D. (1993). Sites of cone system sensitivity loss in retinitis pigmentosa. *Investigative Ophthalmology and Visual Science, 34*, 2638–2645.

Seiple, W., Siegel, I., Carr, R. & Mayron, C. (1986). Evaluating macular function using the focal ERG. *Investigative Ophthalmology and Visual Science, 27*, 1123–1130.

Sutter, E. E. (1991). The fast m-transform: a fast computation of cross-correlations with binary m-sequences. *Society for Industrial and Applied Mathematics, 20*, 686–694.

Sutter, E. E. & Tran, D. (1992). The field topography of ERG components in man—I. The photopic luminance response. *Vision Research, 32*, 433–466.

Verdon, W. & Haegerstron-Portnoy, G. (1996). The normal multifocal electroretinogram. *Optometry and Vision Science, 73*, 83.

Wu, S. & Sutter, E. E. (1995). A topographic study of oscillatory potentials in man. *Visual Neuroscience, 12*, 1013–1025.

Yagasaki, K., Jacobson, S. G., Apathy, P. P. & Knighton, R. W. (1988). Rod and cone psychophysics and electroretinography: methods for comparison in retinal degenerations. *Documenta Ophthalmologica, 69*, 119–130.

Acknowledgements—Supported by National Eye Institute grants R01-EY-09076 and R01-EY-02115, a grant from The Foundation to Fight Blindness to NYU Medial Center, and a grant from The Macula Foundation, Inc. We thank S. Shady for his helpful comments. Preliminary versions of these findings were reported at the meetings of ARVO (1996), ISCEV in Tübingen (1996) and VSIA (OSA-1997).