Comparison of Fundus Autofluorescence with Photopic and Scotopic Fine-Matrix Mapping in Patients with Retinitis Pigmentosa and Normal Visual Acuity

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PURPOSE. To compare psychophysically determined spatial variations in photopic and scotopic sensitivity across the macula in patients with retinitis pigmentosa (RP) and normal visual acuity who manifest an abnormal high-density ring of fundus autofluorescence (AF).

METHODS. Eleven patients with a clinical diagnosis of RP were examined. All had rod-cone dystrophy (International Society for Clinical Electrophysiology of Vision [ISCEV]-standard ERGs), visual acuity of 6/9 or better, and an abnormal parafoveal annulus of high density AF. Fine-matrix mapping (FMM) was performed over macular areas of abnormal high-density AF under photopic and dark-adapted conditions. Pattern ERGs (PERGs) were performed in 9 of 11 patients, by using different sizes of circular checkerboards.

RESULTS. Rings of high-density AF varied between patients (approximately 3°–18° in diameter). Photopic sensitivity was preserved over central macular areas, but there was a gradient of sensitivity loss over high-density segments of the ring and severe threshold elevation outside the arc of the ring. Scotopic sensitivity losses were more severe, and they encroached on areas within the ring. The radius of the high-density ring correlated with the lateral extent of preserved photopic sensitivity (r = 0.86) and PERG data.

CONCLUSIONS. High-density rings of AF, which are present in some patients with RP with normal visual acuity, demarcate areas of preserved central photopic sensitivity. Scotopic sensitivity losses encroach on areas within the ring of high density and may reflect dysfunction before accumulation of lipofuscin.

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I n vivo autofluorescence (AF) of human pigment epithelium has provided a useful tool in the investigation of inherited and acquired retinal disease,1–8 allowing visualization of lipofuscin at the level of the RPE.1,3,9 The absence of AF may reflect photoreceptor cell death, leading to atrophy of the RPE. However, the presence of increased lipofuscin suggests continuing metabolic demand1 possibly representing compromised RPE function10 without or before photoreceptor degeneration.

Our experience with AF imaging is that some patients with retinitis pigmentosa (RP) and normal visual acuity have abnormal fundus AF in the form of a parfoveal ring of high density.11 This initial study revealed that the size of the high-density ring is proportional to the amplitude of the pattern ERG (PERG) P50 component, indicating differing degrees of macular cone-system involvement.12 The object of the present study was to compare psychophysically determined spatial variations in both photopic and scotopic thresholds with the distribution of these fundus AF abnormalities and to establish their significance in terms of both cone and rod system function.

METHODS

The protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Eleven patients, who formed part of a previously described cohort of 30 patients,11 were re-examined, by using detailed psychophysical techniques, and results compared with those obtained in a normal group of 10 subjects. The mean and median ages of the 11 patients were 32.1 ± 9.2 and 32.0 years, respectively. The mean and median ages of the 10 normal subjects were 35.7 ± 9.3 and 32.0 years, respectively. Further electrophysiological examination was performed on the patient group. All had a Snellen visual acuity of 6/9 or better (seven had a visual acuity of 6/6-2 or better in both eyes), a clinical diagnosis of RP, International Society for Clinical Electrophysiology of Vision (ISCEV)-standard full-field ERGs consistent with rod-cone dystrophy, and abnormal AF of the posterior pole in the form of a high-density parfoveal ring. AF imaging was performed with a prototype system (SM 50-4024; Carl Zeiss Meditec, Oberkochen, Germany) confocal scanning laser ophthalmoscope (cSLO), according to previously described techniques.1,2,13 The fundus was illuminated with argon laser radiation (488 nm, 250 µW). The induced AF was viewed through a long-pass filter with a short-wavelength cutoff (50% transmission at 521 nm, 1 × 10^-3 transmission at 495 nm; Coherent-Ealing Europe Ltd., Leicester, UK; with an OG515 filter; Schott, Duryea, PA). The cSLO images were recorded at standard video scanning rates and digitized at 256 × 256 resolution. Thirty-two individual images of each fundus were digitized at six frames per second and aligned and averaged to reduce noise.

The topographic distribution of AF across the retina was measured with a grayscale index of intensity (0–255 units), along the vertical and horizontal meridians, intersecting the foveal pixel,14 and scaled in degrees by assuming a visual angle of 15° between the optic disc and fovea.

Photopic fine-matrix mapping was performed.15–18 For photopic testing, we used standard size-3 target flashes (subtending a visual angle of approximately 0.51°) presented randomly at 1° intervals within a 9° × 9° matrix, with a modified Humphrey perimeter (background illumination 31.5 apostilbs [asb]; Carl Zeiss Meditec). Subjects signaled detection thresholds with a push-button control. Test flashes were positioned over the retinal area of interest, usually covering areas of abnormal AF and aligned according to the position of the blind spot, located psychophysically. In most subjects, two 9° × 9° matrices were used to measure sensitivity over larger retinal areas. Four red-light-emitting diodes arranged in a diamond configuration were used to aid central fixation, monitored using closed-circuit television. Observers were instructed to maintain fixation on a central point equidistant from each of the surrounding LEDs. Detection sensitivity was expressed in
decibels and thresholds as log units (4 log units = 10,000 asb). Sensitivities were shown as contour plots, illustrating the position and orientation of test matrices, and as three-dimensional threshold profiles, plotted by using interpolated values at 0.25° intervals, obtained by Gaussian filtering.19,20

Scotopic fine-matrix mapping was performed with blue test stimuli (size-3 target) in six of the same subjects over identical retinal locations after pupil dilation (tropicamide 1%) and 40 minutes of dark adaptation. The radius of the high-density ring was compared with the eccentricities at which photopic sensitivity was reduced to 25 dB or less (threshold ≤ 1.5 log units or 32 asb), and scotopic sensitivity was reduced to 5 dB or less (threshold ≤ 3.5 log units).

Each 9° × 9° fine-matrix map took the patient approximately 40 minutes to perform, excluding time for dark adaptation. Normal values were obtained for a central macular area and for a single paracentral location under photopic (10 subjects) and scotopic (8 subjects) conditions.

PERGs were recorded in 9 of 11 patients. Responses were evoked using high-contrast checkerboard reversal, with gold-foil recording electrodes, according to ISCEV standards21: reversal rate 2.2 Hz, checkerboard size 12° × 15°, check size 45 minarc, mean luminance 50 cd/m², and Michelson contrast 0.98. Recordings were performed binocularly to facilitate optimal fixation. Typically, more than 100 sweeps were averaged, using “interrupted stimulation” to minimize eye movements. Patients were instructed to concentrate on a small fixation spot centered on the stimulus. Averaging was then suspended every 4 to 5 seconds, and the patients were told to blink before resuming fixation.

The PERG P50 component was used as an index of macular function. Additional PERG testing was performed with circular fields of different diameters (5°, 6°, 9°, 12°, and 18°) presented to each patient in random sequence, with check size constant at 45 or 23 minarc.

RESULTS

Figure 1 shows examples of full-field ERGs, PERGs, and AF images from three representative patients (Figs. 1A–C) and in a normal subject (Fig. 1D). The full-field ERGs in the patients are consistent with rod–cone dystrophy and the clinical diagnosis of RP. In the 11 patients tested psychophysically in this study, PERG P50 components varied in amplitude from normal (≥ 2.0 μV) to undetectable, suggesting different degrees of macular involvement. As an inclusion criterion, all patients had a parafoveal ring of high density (Fig. 1, right column). The internal radius of the high-density ring showed a high degree of interocular symmetry (r = 0.97, N = 11) and intersubject variability. The internal diameter of the ring varied between approximately 3° and 18°.

Figure 2 shows examples of contour plots obtained in 1 normal subject at a central (Figs. 2A, 2B) and a paracentral (Figs. 2C, 2D) retinal location under photopic (Figs. 2A, 2C) and scotopic (Figs. 2B, 2D) conditions. Three-dimensional threshold plots represent mean values obtained at corresponding retinal locations under photopic (10 subjects) and scotopic (8 subjects) conditions. A small increase in photopic sensitivity at the fovea was present in some subjects, but was not clearly seen after between-subject averaging (Fig. 2A, see the Discussion section). Mean photopic sensitivity showed minimal variation over the central area, with mean thresholds increasing by approximately 0.5 log units at the most eccentric location tested (Fig. 2C, location a).

Fine-matrix mapping under scotopic conditions (N = 8) revealed low sensitivity at the fovea (Fig. 2B), consistent with the normal low density of rod photoreceptors in this area22,23 (see the Discussion section). Mean scotopic thresholds decreased by more than 2 log units between the fovea and outer regions of the central 9° × 9° area (Fig. 2B) and showed minimal further reduction with eccentricity (Fig. 2D). Average thresholds and standard deviations for selected retinal locations are shown in Table 1.

Figures 3 and 4 show representative data from five patients who manifested an abnormal ring of high-density AF. Three-dimensional plots represent threshold elevation above the mean and were obtained by subtracting the mean normal values in Figure 2 from thresholds obtained at identical retinal locations in individual patients. In all subjects, there was relative preservation of photopic sensitivity within the high-density ring, with abnormal elevation of thresholds over the arc of high density (Figs. 3, 4, rows 1 and 2; Fig. 5). This is most clearly seen in a subject with a large, broad ring (Figs. 4B, 4C, rows 1 and 2). Over the central macula of this eye, photopic thresholds were within 0.25 log units (approximately 1 SD of the foveal value) of the normal mean. Contour plots across a segment of high-density AF revealed a gradient of photopic sensitivity loss resulting in a plateau of threshold elevation exceeding 1.5 log units (> 3 SD above the mean) and extending over more eccentric areas outside the arc of high density.
Figure 6 summarizes data from all patients who underwent photopic fine-matrix mapping, by comparing the ring radius with the eccentricity at which sensitivity declined to 25 dB (threshold 1.5 log units or 32 asb), revealing a strong correlation ($r = 0.86, N = 11$). This eccentricity is related to the minimum field size necessary to elicit a maximum amplitude PERG P50 component, as shown in a representative cohort of four patients (Fig. 5). There is high correlation between the minimum field size necessary to elicit a maximum-amplitude PERG P50 component and the mean eccentricity at which photopic thresholds are reduced to 25 dB ($r = 0.74, n = 9$).

The spatial pattern and magnitude of scotopic sensitivity loss was more extensive and threshold elevation greater than that under photopic conditions. Contour and threshold plots in subjects with smaller rings (Fig. 3, rows 3 and 4; Fig. 4A, rows 3 and 4) revealed scotopic sensitivity loss encroaching on central macular areas within the rings of high density. Thresholds became less severely elevated toward the fovea. In a patient with a large, high-density ring (Figs. 4B, 4C, rows 3 and 4), almost all thresholds over the central $9^\circ \times 9^\circ$ were within 0.6 log units (approximately 1.3 SD) of the normal foveal mean (Fig. 4B, row 4). Over more eccentric areas closer to the internal edge of the ring,

![Figure 2](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932923/)
thresholds were elevated by a minimum of 1 log unit (Fig. 4C, row 4). A sharp gradient of sensitivity loss was detected at more peripheral locations, with thresholds increasing from 1 to between approximately 2.5 and 3 log units (>4 SD) above normal, over areas that are concordant with the distribution of the ring. The spatial extent of scotopic sensitivity loss can be related to the size of the high-density rings. The eccentricity at which rod system sensitivity decreased to an arbitrary value of 5 dB (threshold, 3.5 log units, or 3160 asb) declined with decreasing ring radius (Fig. 6, \( r^{0.84} \)).

**DISCUSSION**

This study demonstrated high spatial correlation between areas of reduced photopic and scotopic sensitivity and the annular areas of abnormally increased AF that manifest in some patients with RP and normal visual acuity. The abnormal high-density AF formed a parafoveal ring that encircled areas in which photopic sensitivity was relatively preserved. The pattern of scotopic sensitivity loss was more severe and encroached on areas within the high-density rings (Figs. 3, 4).

Our initial studies, performed on a cohort from a heterogeneous group of patients, demonstrated approximate correspondence between ring size and visual field loss, ascertained using the relatively coarse spatial resolution of automated Humphrey perimetry (Carl Zeiss Meditec).\(^{11}\) The present study confirms and extends that qualitative finding. High-resolution, fine-matrix maps showed correlation between markedly elevated photopic thresholds and the distribution of high-density AF, representing the internal edge of visual field constriction. In addition, fine-matrix mapping revealed a gradient of photopic sensitivity loss over the high-density segments tested (Figs. 3, 4, 5).

The PERG P50 component allows assessment of suprathreshold macular function under photopic conditions, arising

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**TABLE 1. Average Thresholds and Standard Deviations for the Different Retinal Locations and Areas Shown in Figure 2**

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Median</th>
<th>Mean for 9° × 9° Area (SD)</th>
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</thead>
<tbody>
<tr>
<td><strong>Photopic ((N = 10))</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foveal location (Fig. 2A)</td>
<td>0.44 (0.25)</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Eccentric location a (Fig. 2A)</td>
<td>0.63 (0.19)</td>
<td>0.59</td>
<td>0.50 (0.19)</td>
</tr>
<tr>
<td>Eccentric location a (Fig. 2C)</td>
<td>1.03 (0.44)</td>
<td>0.93</td>
<td>0.87 (0.44)</td>
</tr>
<tr>
<td><strong>Scotopic ((N = 8))</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foveal location (Fig. 2B)</td>
<td>3.06 (0.37)</td>
<td>2.93</td>
<td></td>
</tr>
<tr>
<td>Eccentric location a (Fig. 2B)</td>
<td>0.92 (0.45)</td>
<td>0.91</td>
<td>1.60 (0.69)</td>
</tr>
<tr>
<td>Eccentric location a (Fig. 2D)</td>
<td>0.90 (0.75)</td>
<td>0.87</td>
<td>0.86 (0.60)</td>
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</table>
partly in structures anterior in the visual pathway to the retinal ganglion cells\textsuperscript{24} and driven by macular cones.\textsuperscript{12} Small-field PERGs performed in the present study corroborate earlier PERG studies of macular function in which ISCEV-standard PERGs were shown to be linearly related to the size of the high-density annulus.\textsuperscript{11} The spatial distribution of reduced cone system sensitivity revealed by fine-matrix mapping is consistent with the PERG and small-field PERG data (Fig. 5) and suggests minimal contributions from areas surrounding the high-density annulus. Correspondence between PERG and photopic fine-matrix mapping data further confirms the utility of PERG P50 in the objective assessment and quantification of macular cone system dysfunction, as suggested in many previous PERG studies.\textsuperscript{5–8,25–27}

Full-field ERGs under conditions of dark adaptation are dominated by the rod system but with minimal contribution from the macula.\textsuperscript{12,28} Macular rod function cannot be inferred from the PERG\textsuperscript{11,12} or from photopic measures of sensitivity. Fine-matrix maps obtained after dark adaptation allow assessment of rod-system function over macular areas identical with those tested under photopic conditions. The observation of loss of scotopic sensitivity found within the AF ring (Figs. 3, 4) suggests that mild macular photoreceptor dysfunction may precede significant accumulation of lipofuscin and increased AF. Also, early macular rod involvement may occur before significant cone system dysfunction, as has often been noted in the peripheral retina of patients with rod-cone dystrophy.\textsuperscript{29–32}

Slightly increased photopic sensitivity at the fovea was evident in some normal individuals, in keeping with the lower mean thresholds at the fovea compared with a parafoveal location (Table 1). The apparently small magnitude and variability of this effect may be attributable to microsaccades and fine-scale interpolation of data that may mask highly localized sensitivity differences. The mean scotopic threshold at the fovea is approximately 3.1 log units in normal subjects (Table 1). This may reflect detection by the cone system, it falls within the expected range, close to the asymptote of the cone dark-adaptation curve (data not shown). Microsaccades, resulting in stimulus detection outside the rod-free area and/or fine-scale interpolation, are also likely to contribute to the lack of a sharp peak in threshold over the central rod-free area (Fig. 2B).

Sensitivity or response amplitude to an increment of light depends on the background level of illumination.\textsuperscript{33–35} There is evidence in RP that variations in sensitivity over a range of adaptation levels may reflect the retinal locus and mechanism of dysfunction.\textsuperscript{36} For example, if photopic sensitivity loss results from a reduction in photopigment density, it would reduce the quantal catch and decrease the effective intensity of both the stimulus and the photopic background.\textsuperscript{37} Thresholds in such a case would be elevated at lower background levels and would approach normality at high photopic levels, as shown in some patients with RP.\textsuperscript{36–38} In the present study, photopic function appeared to be normal over central areas of macula, consistent with small-field PERGs recorded over similar areas (Fig. 5) and with preliminary studies of multifocal ERGs that reveal preservation of central but not paracentral responses (Robson AG, et al. IOVS 2003;44:ARVO E-Abstract 535). Photopic sensitivity losses over more eccentric areas were milder than under scotopic conditions, in keeping with full-field ERGs that show peripheral involvement of rod more than cone photoreceptors.\textsuperscript{11} However, additional psychophysical stud-
Fundus AF is driven by rod photoreceptor outer segment turnover. Retained or slightly elevated levels of AF, present outside the ring in most patients, suggest continuing metabolic demand from rods that are intact but dysfunctional. This is in keeping with the type 1 or “diffuse” form of RP, typically associated with sensitivity loss in the presence of high levels of rhodopsin. One implication is that intact photoreceptors may in future be amenable to functional rescue, as shown in recent animal models of RPE65.

In one patient, there was a mottled or discontinuous appearance of AF outside the ring (Fig. 5A), accompanied by mild central sensitivity loss. In another patient, there were low-density areas outside the vascular arcades, consistent with patches of atrophy (Fig. 3C, peripheral areas not shown). The presence of such functional and degenerative changes may reflect different stages of the disease or a different functional subtype (e.g., Type 2 or the “regional” form of RP). The high-density ring may be a nonspecific manifestation.

It is tempting to speculate that the high-density ring may be a relatively late manifestation of slowly advancing RP and that progressive visual field loss may mirror constriction of the AF ring, led by encroaching rod dysfunction over more central and concentric macular areas. As the ring becomes smaller there is additional cone dysfunction over central areas, eventually resulting in reduced central acuity. Longitudinal studies encompassing cases in which there has been visual acuity reduction are needed to investigate this proposal.
CONCLUSIONS

Suprathreshold and threshold measures of macular function suggest that the high-density rings of increased AF that are present in some patients with RP, are of functional significance. They demarcate central areas of largely preserved sensitivity and correspond with the internal edge of photopic visual field constriction, as measured using high-resolution fine-matrix mapping. The presence of normal or slightly increased AF in surrounding areas suggests the presence of intact photoreceptors. Scotopic sensitivity losses encroach on areas within the ring of high density and may reflect dysfunction before accumulation of lipofuscin.

References