Foveal Cone Involvement in Retinitis Pigmentosa Progression Assessed Through Flash-on-Flash Parameters

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**Purpose.** To compare psychophysical Naka-Rushton parameters in retinitis pigmentosa (RP) patients and healthy controls using a flash-on-flash increment threshold paradigm, and to measure changes of these parameters with RP progression.

**Methods.** Sixty-six RP patients and 10 normal subjects were tested, and their maximum response ($R_{\text{max}}$), half-saturation intensity ($\alpha$), and slope ($n$) parameters were estimated.

**Results/Conclusions.** $R_{\text{max}}$ in RP patients is decreased significantly with respect to the range in normal controls and continues to decrease (0.024 log units/yr) with disease progression. The distribution of $\alpha$ in RP patients differs from that in normal subjects, showing lower values in general, but no progression. Small differences in parameter distributions among genetic or pathophysiologic RP subcategorizations were found, but these do not fulfill stricter statistical criteria required for multiple comparisons. Measurement noise, inherent in the flash-on-flash paradigm, exert considerable influence on the quality of the data, as was demonstrated through repeated measures and a Monte Carlo simulation. Invest Ophthalmol Vis Sci. 1993;34:231-242.

Typical retinitis pigmentosa (RP) is a progressive pigmentary retinal degeneration that begins in the midperiphery and eventually spreads to involve the far peripheral retina and the central retina. Although all genetic modes of inheritance are represented among RP patients, 50% of the patients have no family history of RP (ie, simplex).1 There are at least two forms of autosomal dominant RP and two forms of autosomal recessive RP.2 The loss of cone system function in the peripheral retina, as measured by the area of seeing in photopic kinetic perimetry, follows an exponential decay. The average time constant (for 1/e loss in visual field area) is 7.4 yr. There are no statistically significant differences in time constant distributions among the different genetic types of RP. However, these RP subtypes differ in the age of visual field loss onset (ie, critical age).3

Visual acuity usually is not significantly affected until late in the course of RP progression.4-6 However, several studies have shown that foveal cone function can be abnormal early in the disease.7-9 In addition, a recent histopathologic study demonstrated reduced density of foveal cone photoreceptors in a young dominant RP eye from a patient with reportedly normal visual acuity.10

Greenstein, Hood, and their colleagues, in a series of studies that employed measures of increment threshold sensitivity to flashed test stimuli on flashed backgrounds, reported decreased response amplitude of the foveal cone system in RP patients. This conclusion was drawn from a nonlinear model for the cone system response—namely, the Naka-Rushton equation, which predicts the basic form of flash-on-flash
increment threshold data. The Naka-Rushton model generates a saturating response versus intensity function defined by three parameters: (1) \( R_{\text{max}} \), the response asymptote; (2) \( \sigma \), the half-saturation intensity; and (3) \( n \), the slope of the normalized function at the half-maximum point. Based on qualitative curve fitting, Greenstein et al concluded that \( R_{\text{max}} \) is reduced in RP, but \( \sigma \) and \( n \) are normal. In a more rigorous re-analysis of their data, Massof et al confirmed that \( R_{\text{max}} \) is reduced and \( n \) is normal, but they showed \( \sigma \) is significantly reduced in RP relative to normal.

Preliminary studies in our laboratory also have indicated that the maximum response level and the semi-saturation intensity in RP patients differ significantly from those in healthy control subjects. A highly significant decrease of the maximum response level with RP progression could be demonstrated, but no significant further change in the semi-saturation intensity seemed to occur.

This report addresses, in a larger test population and in greater detail, the significance of foveal flash-on-flash parameter changes in RP. While confirming our earlier findings, we provide evidence that the absence of significant further change in semi-saturation intensity with RP progression holds up on a larger sample. We further demonstrate that the pathophysiologic or genetic variety of RP has little influence on these measures. Finally, we show that the complexity of the experimental paradigm and the estimation technique of the Naka-Rushton parameters through a least-square fitting procedure seriously hamper the applicability of flash-on-flash as a standard clinical test.

In an accompanying report in this issue we present foveal impulse response parameters obtained in a parallel test paradigm in the same population, and argue that those parameters provide information that confirm the results extracted from flash-on-flash parameters, and do so with greater accuracy.

**MATERIALS AND METHODS**

**Subjects**

Data were collected from 66 typical RP patients, representing pathophysiologic subtypes and various modes of inheritance. Table 1 gives a breakdown of patient numbers in different categories. Patients whose pathophysiologic subtype could not be established represent those with X-linked inheritance and those whose disease progression was so far advanced that the standard two-color absolute threshold perimetry test used for subtyping was inconclusive. Control data were collected from 10 healthy adult observers. All subjects had visual acuity 20/40 or better in the tested eye, and most had 20/25 or better without correction. In 10 RP patients and 1 healthy control subject, the test was repeated a year or more after the initial administration. These repeated measurements are included in the data presented here, but have been treated separately in the statistical analysis.

**Table 1. Distribution of Pathophysiologic Subtype and Mode of Inheritance Among the 66 Typical RP Patients Tested in This Study**

<table>
<thead>
<tr>
<th>Subtype 1</th>
<th>Subtype 2</th>
<th>Unknown Subtype</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant</td>
<td>8</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>X-linked</td>
<td>—</td>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>Multiplex</td>
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<tr>
<td>Simplex</td>
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<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>29</td>
<td>66</td>
</tr>
</tbody>
</table>

**Procedure**

Stimuli were presented in Maxwellian view in a triple beam optical apparatus. A bite bar was used to steady the subject's head position. Beam diameter at the plane of the pupil was approximately 1 mm; natural, undilated pupil viewing was used. All lights were broadband yellow (tungsten with Kodak [Rochester, NY] 12 Wratten filter). Background light and all stimuli were presented in central fixation, provided by a black crosshair with 2° gaps in the center. A 500 td (photopic troland) circular background field with 5.2° diameter formed the adapting background. A circular pedestal flash of 500 ms duration and 1.3° diameter and a circular test flash with 0.5° diameter and 50 ms duration were presented repetitively. The subject used a push-button to indicate whether the test flash was perceived. The difference between test and pedestal flash duration was chosen to prevent detection of the after-image rather than of the test flash itself. Repetition intervals ranged from 3–10 sec and were lengthened as pedestal and test flashes increased in brightness to minimize changes in the state of adaptation. Test flash intensities ranged from threshold (typically 100–500 td) to 2.4 × 10^6 td (the maximum attainable with our apparatus), whereas pedestal flash intensities ranged from threshold (typically 5–25 td) to 1.5 × 10^5 td. The intensity of the pedestal flash was increased in 0.5 log unit (0.25 log units at intensities over 25,000 td) intervals, and three downward threshold crossings in a staircase procedure were obtained at every pedestal intensity. Figure 1 shows the stimulus timing and layout as seen by the observer.

Data from this test can be plotted as test flash intensity versus pedestal flash intensity. This has been
FIGURE 1. Schematic representation of the stimulus used in our flash-on-flash experiment, showing the spatial arrangement, and, at the bottom, the timing of pedestal and test flashes.

done in Figure 2a for a representative group of patients and for a normal observer, with pedestal and test flash intensities expressed in log units relative to the maximum levels obtainable in our apparatus. Figure 2b shows another typical data set for a normal observer, along with the parametric fit derived.

The procedures followed the tenets of the Declaration of Helsinki. Subjects were carefully instructed regarding the purpose and method of the test, which was part of their participation in a longitudinal study of the natural course of RP. They signed a consent form—approved by the institution’s Joint Committee on Clinical Investigation—to participate in the study.

Analysis

A theoretical justification of the analysis used is given elsewhere. Here, we will merely summarize the derivation of the parametric fit. Values for the maximum response $R_{\text{max}}$, semi-saturation intensity $\sigma$, and slope $n$ of the psychophysical Naka-Rushton function

$$ R = \frac{R_{\text{max}}I^n}{I^n + \sigma^n} \tag{1} $$

were estimated from a set of test flash threshold values $\Delta I$, representing the minimum intensity needed to reach a constant internal incremental response threshold $\Delta R$ on different pedestal intensities $I$. Because $\Delta R$ is small, the first-order approximation can be used:

$$ \Delta I = \Delta R \frac{dR}{dl} \tag{2} $$

FIGURE 2. (a) Representative flash-on-flash data from seven RP patients and one normal observer plotted on a log-log scale. Lines have been drawn for viewing ease only. The bottom/rightmost data set is that for the normal observer. (b) Two least-squares fits obtained with equation (3) in the text. The slope parameter was kept fixed for the fit marked "$n = 1$."
Thus, simultaneous parameter estimation was per-
dance with the second term of the same equation. The
of the high-intensity asymptote equals 2, in accor-
the fifth term in shown. Note that for this value of
n, fit, with the slope parameter fixed at 1.0, also is
formed through a least-squares fit of this form, using a
or, replacing log(A/?) by a constant k,
log(Δf) = k + 2 log(I^n + σ^n)
- log(R_max^n) - n logσ - (n - 1) logI.  (3)

Thus, simultaneous parameter estimation was per-
formed through a least-squares fit of this form, using a
simplex algorithm. The resulting fit to the data of a
normal observer can be seen in Figure 2b. A second
fit, with the slope parameter n fixed at 1.0, also is
shown. Note that for this value of n, the fifth term in
the right hand side of equation (3) vanishes. Thus, the
low-intensity asymptote is horizontal, as determined
by the first, third, and fourth terms, whereas the slope
of the high-intensity asymptote equals 2, in accor-
dance with the second term of the same equation. The
rightmost data point seems to exceed the asymptotic
fit. This phenomenon, observed in most flash-on-flash
data sets, indicates there is a high-intensity limit to the
validity of the linear approximation used in equa-
tion (2).

It has been shown\textsuperscript{17} that a reliable fit depends on
the range spanned by the data points, as well as on the
number of data points. In the present study, only data
that spanned at least 1.5 log units (3–5 data points) of
pedestal flash intensities on either side of the steepest
curvature in the resulting fit were accepted into the
final analysis. Thus, at least 7 data points were in-
cluded in each fit, and this number typically was 8–11.
Patient data, although not always as tightly clustered as
the normal data shown in Figure 2b, could be fitted
without difficulty using the model of equation (3).

Statistical comparisons of the parameter values
for normal observers versus RP patients, and between
subgroups of RP patients, have been made with non-
parametric tests (Mann-Whitney U test for two-way
analysis; Kruskal-Wallis test for four-way analysis), be-
cause of the relatively small number of subjects in each
subgroup and because parameters may not have been
normally distributed. Statistical tests were performed
at three levels. Initial tests were performed to establish
main effects—ie, distinctions between parameter dis-
tributions in patients and normal observers, parameter
dependence on disease progression, and correla-
tions among the Naka-Rushton parameters. Subse-
quently, we tested for possible distinctions among
different RP subcategories. The null hypothesis in
these tests was that—similar to other measures, such
as visual field loss—flash-on-flash parameters do not
show a distinction according to the patient’s patho-
physiologic subtype or mode of inheritance. Finally,
we performed Monte Carlo simulations to determine
if, and to what extent, our results could be influenced
by measurement noise.

The “independent” parameter in our study—ie,
time past critical age—was distributed widely, as can
be seen in Table 2. Only minor differences in distribu-
tion of this parameter between subtypes were found,
but a marginally significant difference was present
among modes of inheritance: Time of progression was
shorter (U = 301.5, n = 62; Z = -1.78, P = .073)* in
patients with dominant and X-linked inheritance than
in patients with multiplex and simplex inheritance (to
be referred to jointly as “recessive” patients in the
remainder of this article). This means that all compu-
tations regarding changes of parameters initially can
be carried out for the entire group of patients, but that
differences between dominant and X-linked patients
on the one hand, and recessive patients on the other,
may have to be corrected for disease progression.

**RESULTS**

Naka-Rushton Parameters: RP Versus
Normal Observers

Means and standard deviations of the parameter dis-
tributions across normal observers and RP patients
and in various RP subpopulations are given in Table 2.

\* The value of Z is a parametric test equivalent to the U score
in the Mann-Whitney test. This value allows a more direct probabil-
ity estimate if at least one of the two groups has more than 20
samples.
It is immediately obvious that, in all subgroups considered, there was a large spread in parameter values, but that the spread was larger among patients than among normal observers.

The maximum responsiveness, $R_{\text{max}}$, exhibited a highly significant ($U = 33, n = 76; Z = -4.56, P < 10^{-4}$) difference between normal and patient values. The semi-saturation intensity, $\sigma$, also showed a difference between the distributions for normal subjects and RP patients, but this difference was only marginally significant ($U = 206, n = 76; Z = -1.90, P \approx .055$). The distribution of the slope parameter, $n$, does not show a significant difference between normal subjects and patients ($U = 220.5, N = 76; Z = -1.68, P \approx .09$). In fact, there were considerable amounts of overlap of the $\sigma$ and $n$ parameter distributions for normal observers and patients. Only for the $R_{\text{max}}$ parameter were the distributions well segregated.

Figure 3 shows the Naka-Rushton functions for the average normal and RP observer, indicating the variability of $R_{\text{max}}$ and $\sigma$ through error bars around the semi-saturation point. Note that these would have been the functions recorded if a single flash on a 500 td background had been used and if there were a way to perform magnitude estimates in this type of experiment. That option being barred, they were derived from the fit in equation (3), as shown in Figure 2.

This table and figure reconfirm our earlier findings\(^1\)\(^2\)\(^3\)\(^4\)\(^5\) that having RP is associated with an important reduction in foveal flash response amplitude and that foveal semi-saturation tends to occur at lower pedestal flash levels in RP patients than in normal observers. However, this does not address the question about whether these foveal parameters already are affected in patients whose disease onset, as judged by visual field progression, took place only recently. Keep in mind that foveal vision, as exemplified by the visual acuity of our subjects (and of RP patients in general), is not usually affected until much later in the course of the disease\(^6\). Therefore, it is interesting to look at Naka-Rushton parameter development as a function of disease progression.

**Naka-Rushton Parameters and RP Progression**

Figures 4–6 show the Naka-Rushton parameters, derived from flash-on-flash data in normal observers and RP patients, and their change with disease progression. This progression is expressed in years past critical age. Figure 4 presents the maximum response amplitude, Figure 5 presents the half-saturation intensity, and Figure 6 presents the slope parameter. Within each figure, the “a” panel shows a break-down of patient data according to pathophysiologic subtype, whereas the “b” panel shows a break-down of the same data according to mode of inheritance. Normal data have been plotted with an abscissa value of 0. Filled symbols for normal data in Figures 4 and 5 indicate that the values of these parameters in patients are significantly different from those in normal subjects.

Regression lines have been drawn through the RP data to indicate significant changes with disease progression, regardless of subcategorizations. It should be emphasized that all RP data (but no normal data) were used in the regression, not just those from patients to whom a pathophysiologic subtype or mode of inheritance could be assigned.

The maximum response parameter, $R_{\text{max}}$, plotted as its logarithm (Figure 4) to conform with the least-squares equation (3), shows a significant ($n = 66, \rho = -0.43; P < 10^{-9}$) dependence on RP progression ($-6.2\%$/year) when all patient data are lumped together. The semi-saturation intensity, likewise plotted as its logarithm (Fig. 5), does not show significant changes in $\log(\sigma)$ with disease progression ($\rho = -0.00$). Similarly, the distribution of the slope parameter, $n$, plotted in Figure 6, only shows a mild trend ($0.0012$/yr) toward slightly larger values in advanced cases of RP, but this trend lacks significance ($\rho = 0.18, P \approx 0.1$).

**Dependence of Parameter Distributions on RP Subcategories**

Through the use of different symbols in Figures 4–6, the influence of RP pathophysiology and the genetic...
In this comparison, we found no significant differences in the distribution of \( \log(R_{\text{max}}) \) for single pathophysiologic subtypes or modes of inheritance. However, the change with disease progression was

\[ \rho = -0.00 \]

**FIGURE 4.** Maximum response parameter, \( \log R_{\text{max}} \), as a function of RP progression. Normal values have been plotted at progression time 0. In (a) of this and following figures, data have been differentiated according to patients' pathophysiologic subtypes, and in (b) according to their inheritance patterns. Filled symbols for normal subjects indicate a significant difference between normal and patient parameter distributions, and regression lines indicate a significant parameter change with disease progression (excluding healthy controls).

inheritance pattern on Naka-Rushton parameter values can be seen. We tested the parameter distributions for possible distinctions using the Mann-Whitney and

**FIGURE 5.** The half-saturation intensity parameter \( \sigma \), plotted logarithmically as a function of RP progression.

Kruskal-Wallis tests. In this process, several tests were performed on each of three parameter distributions. Because within each parameter set these tests were performed on the same sample of parameter values, it seems prudent to require stricter \( P \) values to establish significance.
stronger in multiplex and simplex RP than in dominant and X-linked RP, and stronger in pathophysiologic subtype 2 than in subtype 1. For a combined dominant/X-linked data subset, the distribution of log($R_{\text{max}}$) values differed significantly (U = 11, n = 30; Z = -3.92, $P < 10^{-4}$) from that in normal subjects, whereas there was no significant dependence (n = 20, $\rho = -0.13$) on disease progression in the two recessive subsets, on the other hand, there was a distribution that was significantly different (U = 23, n = 52; Z = -4.34, $P < 10^{-4}$) from that in normal subjects, and a highly significant decrease with RP progression (n = 42, $\rho = -0.47$, $P < 10^{-3}$). Similarly, the log($R_{\text{max}}$) distribution in subtype 1 patients was significantly different from that in normal subjects (U = 11, N = 27; Z = -3.72, $P < 10^{-3}$), but did not change significantly with disease progression (n = 17, $\rho = -0.13$). However, in subtype 2, log($R_{\text{max}}$) was not only significantly smaller than in normals (U = 13, n = 39; Z = -4.25, $P < 10^{-4}$), but also showed significant further decrease with RP progression (n = 29, $\rho = -0.43$, $P \approx 0.02$). The lack of significance of two correlation coefficients (−0.13) cannot be attributed only to the smaller sample sizes. $\rho$ values in the two subsets concerned were much smaller than those in the other two sets, even if both distributions show a trend in the proper direction.

For the semi-saturation intensity $\sigma$, no significant difference was found between pathophysiologic subtypes (U = 168, n = 46; Z = −1.79); a slight difference among modes of inheritance also fails to attain significance by our stricter criterion. Log($\sigma$) is smaller for the combined recessive subsets than for the combined dominant and X-linked subsets, but only marginally so (U = 299, n = 62; Z = −1.82, $P \approx 0.07$). Therefore, RP subcategories could not be distinguished on the basis of their log($\sigma$) distributions. Incidentally, $\sigma$ values in type 1 and in dominant/X-linked RP patients showed no significant difference from those in normals ($Z = -0.70$ and $-0.35$, respectively), whereas significant differences from normals were found in type 2 and recessive patients ($Z = -2.57$, $P \approx 0.01$ and $Z = -2.21$, $P \approx 0.03$, respectively). However, because we have already established the main effect of log($\sigma$) difference between normal subjects and RP patients, we must assume that all RP patients have log($\sigma$) values different from normals. Furthermore, analysis of the data in Figure 5 for effects of RP progression did not yield significant changes in log($\sigma$) with disease progression for either subtype ($\rho = -0.14$ in both cases) or for modes of inheritance ($\rho = 0.07$ for the dominant/X-linked; $\rho = 0.06$ for the recessive data subsets).

Similarly, no distinctions according to subtype or mode of inheritance could be demonstrated for the slope parameter, $n$, in overall distributions or in changes with RP progression.

**Correlations Among Naka-Rushton Parameters**

In Figures 7–9, correlations among the Naka-Rushton parameters were plotted, with mode of inheritance as a parameter. Regression lines again have been drawn in cases where significant pairwise correlations of parameters were found. Normal data, represented by solid symbols only where significantly different along

![FIGURE 6. The slope parameter, $n$, plotted as a function of RP progression.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933395/)
FIGURE 7. Covariance of \( \log(R_{\text{max}}) \) and \( \log(\sigma) \), and the significant bivariate regression line of this parameter distribution.

FIGURE 8. Covariance of \( \log(R_{\text{max}}) \) and \( n \).

FIGURE 9. Covariance of \( \log(\sigma) \) and \( n \).

both axes, were not included in computation of the correlations and regression lines.

Figure 7 shows that there was a highly significant \((\rho = 0.43; P < 10^{-3})\) correlation of changes in \( \log(R_{\text{max}}) \) and \( \log(\sigma) \). Distributions were similar for subsets of the data, but because of smaller sample sizes, not all correlation coefficients were significant. For subtype 1, \( p = 0.48 \) (n = 17, \( P = 0.05 \)); for subtype 2, \( p = 0.39 \) (n = 29, \( P < .05 \)); for dominant/X-linked inheritance, \( p = 0.34 \) (n = 20, ns); and for recessive inheritance, \( p = 0.29 \) (n = 42, \( P = .01 \)). This similarity supports the notion that there are no significant distinctions according to inheritance pattern or pathophysiologic subtype in the \( \log(R_{\text{max}}) \) versus \( \log(\sigma) \) relationship.

The correlation of \( \log(R_{\text{max}}) \) and \( n \) (Fig. 8) is not significant, and no significant differences were found according to the pathophysiology or mode of inheritance of the disease.

Similarly, the correlation of \( \log(\sigma) \) and \( n \) (Fig. 9) is not significant, and no significant distinctions can be made according to inheritance pattern or RP pathophysiology.

DISCUSSION

Flash-on-Flash Test Reliability

Our results suggest that RP patients show significant differences from normal subjects for two out of three psychophysically determined Naka-Rushton parameters; that significant further changes with progression of the disease occur for the maximum response parameter only; and that the only significant differences among subsets of patient parameters are those in \( \log(R_{\text{max}}) \) change with RP progression, between patients with dominant and X-linked inheritance, and those with recessive (multiplex or simplex) inheri-
tance, and similarly between patients with type 1 and those with type 2 RP.

Admittedly, some of the differences found—notably that of log(σ) between normals and patients—are not very strong, and major contributions to the dependent parameter values (other than RP progression) remain unaccounted for. Therefore, before any further interpretation of the data is attempted, it seems useful to look at the reproducibility of parameter measurements and at possible sources of spurious correlation. We looked at correlations through a Monte Carlo simulation, whereas reproducibility was tested through repeated measurements obtained in 10 patients and 1 normal subject.

Monte Carlo Simulation

The problem of parameter interactions is inherent in our analysis. Three parameters estimated from a two-dimensional fit cannot be independent. A change in \( R_{\text{max}} \) corresponds to a vertical shift in the fitted curve, a change in \( \sigma \) corresponds to a shift along a line with slope 1 on a log-log scale, and a change in \( n \) corresponds to shallowing or steepening of the curvature and low- and high-intensity slopes. To obtain a measure of the interactions among parameter estimates, of the influence of measurement noise, and of the confidence intervals around the parameter values, we produced for each subject a new data set with 50 records. To create a record in this set, test flash intensities from the patient’s original record were offset by 0.1 log units (0.3 log units for intensities over \( 10^5 \) td), with random positive or negative sign. These values are typical for curve-fitting deviations observed in our raw data.* Sixty-six data sets were formed. Only patient data were used, and only one data set for each patient was used.

After curve-fitting the 50 records within each data set to obtain 50 parameter triplets, we looked at the following statistics.

Bias. Are the 50 values of each parameter distributed symmetrically around the original value? If not, measurement noise in the original data also would have influenced parameter estimates. We found that in most subjects (78%), all three original parameter values fell within 0.2 standard deviations from the mean of their respective distributions. The offset distributions showed slight asymmetry (\( -0.0029 \pm 0.0040 \) for log(\( R_{\text{max}} \)), \( -0.0066 \pm 0.0114 \) for log(\( \sigma \)) and \( 0.0013 \pm 0.0026 \) for \( n \)), but these are small values compared to the variations found in the normal subject and patient data. It seems, therefore, that the curve fitting procedure is sufficiently insensitive to measurement noise.

Quality of Fit. This refers to the distribution of the total standard deviation (SD\(_{\text{total}}\), the square root of the mean square error) across all 50 fits, and a comparison of this deviation with the root mean square (rms) errors in the original fits (as defined in the footnotes). We found that SD\(_{\text{total}}\) had a mean value of 0.094—ie, similar to the noise levels introduced to create the simulation—and that there was a significant negative correlation (\( \rho = -0.38, P < 10^{-3} \)) of the original rms and SD\(_{\text{total}}\)—ie, the worse the original fit, the less the influence of the data perturbation. Thus, measurement noise in the original data will influence the parameter estimates, but it will not make the fitting procedure diverge.

Interactions. This refers to the pairwise correlations among the parameters obtained from each data set. We found a significant negative correlation of log(\( \sigma \)) and \( n \) in 95% of the data sets, with a mean value of \( \rho = -0.51 (P < 10^{-4}) \), and a slope \( m = -0.15 \) for the regression line through significant values. In only 38% of the data sets was there a significant correlation of log(\( R_{\text{max}} \)) and \( n \), whereas a significant correlation of log(\( R_{\text{max}} \)) and log(\( \sigma \)) was found in 72% of the data sets (\( \rho = 0.28, P < .05, \text{slope} = 1.88 \)). However, if only log(\( R_{\text{max}} \)) values above average were included in the computations, a much stronger correlation with log(\( \sigma \)) was found (\( n = 40, \rho = 0.47, P < 10^{-3} \)), whereas the correlation was negligible for smaller log(\( R_{\text{max}} \)) values. Such range-dependent interactions were not found for the other parameter pairs.

The simulation also was carried out at 50% of the noise level already given. The same correlation values were found. Noise levels in the parameters and in the bias of \( n \) changed proportionally. Bias values for log(\( R_{\text{max}} \)) and log(\( \sigma \)) changed more rapidly, however (approximately as the noise level cubed).

Reproducibility

To test the reliability of the flash-on-flash technique, we compared follow-up visits in individual subjects. If within-observer variations in parameter estimates

* An anonymous reviewer, unconvincing by the significance level of the difference in values between normals and RP patients, suggested the use of an F-test to see whether the two samples could have been drawn from a single distribution. The outcome of this test rejects that notion—\( F(65,9) = 3.66, P < .05 \). Once again, this does not represent a high significance level, but it supports the notion that the half-saturation intensity in RP patients differs from that in normals. Moreover, we previously have found decreased log(\( \sigma \)) values in other RP flash-on-flash data, from our own laboratory and through a retrospective analysis of literature data. The larger variance in patients’ log(\( \sigma \)) distributions can partly account for the marginal significance of the Mann-Whitney test.

These rms values, measuring the quality of fit of a three parameter function to \( N \) data points, were obtained as the square root of \( 1/(N-5) \) times the sum of squared deviations. Note that these values illustrate the difficulty of the procedure for the subject. In fact, normal subjects (rms = 0.21 log units) were slightly worse at setting flash-on-flash thresholds than RP patients (rms = 0.19 log units).
were small compared to the standard deviations in Table 2, we can be confident that parameter differences between subjects represent true inter-individual variability. From the small number of measurements (M = 2) per subject, it obviously is impossible to estimate individual parameter distributions, but if we can correct for the true changes in parameters over the 1–2 yr follow-up time and assume that the noise—responsible for the remaining parameter changes—has the same distribution in every subject, we can estimate the standard deviation of this noise as 1/√2 times the rms across subjects of the parameter difference between visits. In RP patients, this procedure yields estimates of 0.30, 0.33, and 0.021 for log(R_max), log(a), and n, respectively, whereas the values in the single normal subject are 0.092, 0.049, and 0.0057.

One may note that the noise estimates in patients are roughly 70% of the standard deviations in Table 2—ie, they account for 50% of the variance. Therefore, in rough approximation it seems reasonable to estimate that one-half of the variance in the parameter estimates was caused by intra-individual variations; these could have been the result of sensory instability brought on by RP or the result of observer uncertainty related to the difficulty of the flash-on-flash paradigm. The other half of the variance could be related to true interindividual differences.

What do these findings imply for the correlations found in Figures 7–9? The positive correlation in Figure 7 may, at least in part, be attributed to measurement noise. It shows the same type of distinction between the low and high range of R_max found in most of the Monte Carlo data sets. Thus, the true correlation of these two parameters probably is minimal. On the other hand, there may be a hidden positive correlation of log(a) and n in our data, counterbalanced by the noise-driven negative correlation found in the simulation. This positive correlation is of minor interest because neither parameter changed appreciably with RP progression.

Relation of Naka-Rushton Parameter Changes to Photoreceptor Changes

Do these changes in Naka-Rushton parameters make sense in the light of our understanding of RP? A decrease in R_max—related to photoreceptor signal strength—could be explained by thinning and shortening of photoreceptors that have been observed in autopsy retina tissue from RP patients.¹⁰

A decrease in a suggests that saturation is achieved by a smaller amount of light than normal—ie, that RP foveal cones saturate at lower incremental luminances than normal foveal cones. This result suggests an abnormality in cone system gain control or feedback mechanisms. For example, the Naka-Rushton function is the solution at the maximum of the phototransduction rate equation

\[ \frac{dR}{dt} = k_1 n(R_{\text{max}} - R) - k_2 R \]  

(4)

At this maximum, \( \frac{dR}{dt} = 0 \), and thus \( a = k_2/k_1 \). Therefore, if the rate of recovery, \( k_2 \), is abnormally slow relative to the rate of response growth, \( k_1 \), \( a \) will be abnormally low. Saturation thus depends on the equilibrium of the phototransduction process, and a decrease of the recovery rate of this process relative to the excitation rate would increase the degree of excitation for a given test flash intensity, thus lowering \( a \).

Such a shift would make sense if, as has been found for photoreceptor dysplasia in the Irish Setter and the rd mouse, the cyclic guanosine monophosphate level in the human RP retina is elevated.²³,²⁴ This elevation is known to lead to slowing of photoreceptor signals and would agree with an increase of the electroretinogram (ERG) implicit time reported by some investigators.²⁵ Subsequent constancy of \( a \) could mean that simultaneous changes of the excitation and recovery phases of the phototransduction cascade occur in more advanced stages of RP.

An increase of \( n \) could be interpreted as an increased area of integration across photoreceptors. Such an increase would be post-receptoral, and therefore is not very plausible. Also, its effect on a large test flash should be minimal, because integration across photoreceptors can affect only the response along stimulus borders. Thus, it is more likely that the small increase of \( n \) with RP progression, observed in our data, represents a shift in \( a \).

We tested the behavior of \( a \) in isolation by re-running the fitting procedure with \( n \) fixed at 1.0 in equation (3). Under this condition, we did find an upward trend in \( \log(a) \) with RP progression, but the change was small and the correlation was far from significant. Also, the difference between normal and RP distributions of \( \log(a) \) diminishes under this procedure (U = 217, n = 76; Z = -1.74). Thus, elimination of this parameter was not helpful for our analysis.

Do the changes found make sense in the light of RP patients’ complaints? Most RP patients complain about difficulties handling high light levels and sudden changes in illumination, in addition to the more familiar complaints about poor night vision and restricted fields. These difficulties in handling saturating amounts of light agree very well with our finding of a reduced half-saturation intensity, whereas the loss of maximum responsiveness, although probably more easily compensated at later stages in the retina, could aggravate RP patients’ problems at low light levels. Moreover, the reduced response range can be expected to affect signal-to-noise ratios along the visual
pathway, and thus affect performance in a variety of everyday tasks.

**Distinctions According to Subtype and Mode of Inheritance**

Longitudinal study of visual field loss in RP has shown that field loss progresses essentially identically for both pathophysiologic subtypes and for all modes of inheritance. Based on those findings, it has been hypothesized that all RP, as observed clinically, is a common secondary stage of multiple pathologies. It seems puzzling, therefore, that the present data suggest distinctions along both lines of classification.

Log($R_{\text{max}}$) shows only significant loss with disease progression in groups of patients with recessive inheritance or those that belong to subtype 2, and log($\sigma$) is significantly lowered only in patient groups with recessive inheritance or those that belong to subtype 2. Differences between subgroups do not reach statistical significance, however.

On average, as can be seen in Table 1, dominant and X-linked patients in our study passed the critical age for onset of II/4e field loss by 15.2 yr, whereas this value is 19.3 for multiplex and simplex patients. This may be the only factor responsible for the change of log($R_{\text{max}}$) with disease progression in recessive patients. If we recompute the regression for a subset of recessive patients with a similar distribution of the time past critical age, no significant progression is found ($n = 26$, $\rho = -0.09$). Note that this implies that the decrease of log($R_{\text{max}}$) with RP progression takes place primarily in advanced stages of the disease ($\mu_{\text{past}} > 20$ yr). On the other hand, the distinction of log($\sigma$) between normal subjects and dominant and X-linked RP patients is barely influenced by correcting for the difference in time past critical age: $Z = -0.40$ instead of the original $Z = -0.35$. Thus, the difference in times past critical age probably was not the only source for the dichotomy in our test population.

It is possible that the use of a foveal sensitivity measure in the present study brought out a distinction that remains hidden with the peripheral probe of a visual field test. However, given the amount of scatter in the flash-on-flash data, it seems prudent to seek confirmation of such a discrepancy through other measures of foveal function. Such a measure, flicker sensitivity, has been tested on the same group of patients and is reported in another article in this issue.

**Interpretation of Psychophysical Findings**

Can we justify the use of psychophysical techniques for studying abnormal photoreceptor function when it is obvious that later stages in visual sensory processing could keep these abnormalities from becoming apparent and that the difficulty of detection is compounded by inter-individual variability at all stages along the visual pathway? It is obvious that the methodology used here requires the assumption that all stages in the visual system have similar limits in dynamic range, so a change at an early stage is reflected at the perceptual level. However, that psychophysical and performance changes occur in many retinal diseases supports such an assumption. Moreover, RP is a disease in otherwise healthy individuals. Its manifestation is limited to the retina, and there is evidence that functional changes may be restricted to the most distal retinal layers. Major retinal changes, such as bone spicule-like pigmentation, first occur in the mid-periphery, but shortening of photoreceptor outer segments occurs over a much wider range of eccentricities. It has been proposed that the mechanism for such widespread changes involves migration, enlargement, and division of RPE cells in extended areas of retina, as has been observed upon disruption of an RPE cell monolayer in tissue culture. Thus, local sensitivity changes in RP patients, even in retinal areas with normal fundus appearance, may reflect dysfunction at the level of photoreceptor outer segments and RPE cells.

RP patients have notoriously weak luminance ERG responses, even at early stages of the disease. Given this difficulty of obtaining direct measures of retinal function in vivo, it seems important to develop psychophysical tools that are sensitive enough to detect early changes in portions of the retina where no clinical manifestation of the disease is yet present. Especially in the fovea of RP patients, where visual acuity, contrast sensitivity, and color discrimination exhibit values in the normal range many years after the onset of peripheral visual field loss, the development of sensitive psychophysical tests can aid our understanding of disease progression.

**Key Words**

foveal psychophysics, increment threshold, Naka-Rushton parameters, retinitis pigmentosa

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**References**


