Comparison of three methods of estimating the parameters of the Naka-Rushton equation

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Abstract. The Naka-Rushton equation empirically describes the amplitude R of the dark-adapted electroretinogram b-wave, as a function of stimulus luminance L, as $R/R_{max} = L^n /(L^n + K^n)$. Estimating the three parameters R_{max}, n, and K of this function from electroretinogram data is of both experimental and clinical interest. Several different approaches have been developed to accomplish this analysis, but these approaches may derive different estimates of the three parameters. To examine this possibility, we compared the results of three methods of fitting the Naka-Rushton equation to data sets obtained from 30 normal subjects. Two methods were nonlinear curve-fitting programs; the third method involved fitting a regression line to transformed data. The results indicate that solutions provided by these methods have consistent differences, which may be an important consideration when comparing results reported in studies that used different curve-fitting methods.

Introduction

The increase in amplitude of the b-wave of the dark-adapted human electroretinogram (ERG) with increasing stimulus luminance is described empirically by the Naka-Rushton equation:

$$R/R_{max} = L^n/(L^n + K^n) \qquad (1)$$

In this equation, R is the response to a stimulus of luminance L, R_{max} is the asymptotic maximum response amplitude, K is the semisaturation constant or luminance required to elicit a response equal to one-half the amplitude of R_{max}, and n is proportional to the slope of the graph of Equation 1 at the point where the stimulus luminance is taken to be K. In practice, R is plotted as a function of log L, and the graph takes the symmetric shape of the hyperbolic tangent function. Initial models interpreted the parameters R_{max}, n and K as independent measures of responsiveness, retinal homogeneity and sensitivity, respectively [1, 2]. However, more recent models

indicate that the parameters may not be independent [3, 4]. The analysis of luminance-response functions has been applied to the study of several clinical populations [1–8].

At high stimulus luminances, a second limb appears on the luminance-response function that reaches a new asymptotic maximum greater than R_{max} [1, 9, 10]. Since additional parameters are required to fit a curve to the complete luminance-response function, most studies have restricted their analyses to the initial portion of the function. To accomplish this, several approaches have been developed. The solutions derived from these approaches have not been previously compared. However, determining if one approach results in a systematic change in one or more of the parameters of the Naka-Rushton equation would be important in contrasting results obtained in studies that used different analyses. In the present study, we addressed this question by applying three commonly used curve-fitting methods to luminance-response functions obtained from 30 normal human subjects. Two of the methods used here are commercially available non-linear curve-fitting computer programs; the third was a method recently proposed by Aylward [11].

Subjects and methods

Dark-adapted luminance-response functions were obtained from 30 normal subjects, whose results served as a control group in a previous study [12]. The ERGs were recorded with a Burian-Allen contact lens electrode referenced to a forehead electrode; the left earlobe served as ground. A Nicolet Compact Four signal averaging system with an amplifier bandpass of 1-1000 Hz (3 dB down points) was used to obtain recordings, which were stored for later analysis.

White (xenon) stimulus flashes were presented in a Nicolet ganzfeld bowl. Flash luminances were attenuated by means of internal strobe settings and Wratten No.96 neutral-density filters. Flash luminances were calibrated with an EG&G model 550 radiometer equipped with a luminance probe and a flash integrator.

The pupil of the test eye was dilated with 1% tropicamide and 2.5% phenylephrine hydrochloride drops. The test eye was then dark-adapted for at least 30 minutes. Drops of 0.5% proparacaine hydrochloride anesthetized the cornea, and a contact lens was inserted under dim long-wavelength illumination. The luminance-response function was obtained by presenting a series of stimulus flashes, in order of increasing luminance (18 steps between -2.97 and 0.82 log cd second/m^2). At each flash luminance, the response to two flashes presented 15 seconds apart were averaged.

The amplitude of the b-wave was measured from the trough of the a-wave

or, if no a-wave was present, from the baseline to the peak of the most positive deflection.

Curve fitting

Equation 1 was fit to luminance-response functions by three methods, denoted A, B and C. Curve-fitting method A used the computer program NAKA-2, which is a nonlinear curve-fitting algorithm developed by L-K-C Technologies (Gaithersburg, MD) specifically for the b-wave luminance function. This program attempts to identify points in the data corresponding to the second rising limb of the luminance-response curve and to remove them. It does this by iteratively fitting the Naka-Rushton equation as the high-luminance data points are removed until successive fits do not improve.

Curve-fitting method B was described by Aylward [11]. After some algebraic manipulation and taking logarithms, the Naka-Rushton becomes linear in log L. This allows one to estimate n and log K by fitting a straight regression line to the data. This analysis is similar to that used on the Hill equation of respiratory physiology [13]. However, in Aylward's method, one must first estimate R_{max} graphically [11], whereas with the Hill equation this step is unnecessary because the corresponding term is always 1 (100%). There are two linearized forms of the Naka-Rushton equation. The first is that used by Aylward [11]:

$$\log\{(R/R_{max})/[1 - (R/R_{max})]\} = n \log L - n \log K . \tag{2}$$

The second is the corresponding equation for the reciprocals of the arguments of the logarithms in Equation 2:

$$\log[(R_{max}/R) - 1] = -n \log L + n \log K . \tag{3}$$

We used the second form because the quantities on the left-hand side required less computation, lessening the possibility of introducing rounding errors into the calculations.

The steps in Aylward's method are first to identify the second rising limb of the luminance-response curve and to ignore these data for the remainder of the analysis [11]. This step is justified on the grounds that the Naka-Rushton equation does not suffice to describe the complete luminance-response function. Second, R_{max} is estimated graphically as the height of the upper horizontal asymptote of the remaining data. Here it is necessary that R_{max} be strictly greater than all of the response data; otherwise the formula to transform the response data will not work. In this study, we set R_{max} at a value that was 1% greater than the highest amplitude response before the second limb. The third step is to compute the transformed responses, log

$[(R_{max}/R) - 1]$, for each response R. A straight line is fitted by the least-squares method to the transformed response values plotted against the log stimulus luminances. The estimate of n is read as the negative slope of the line and log K as the vertical intercept divided by the estimate of n. The slope and vertical intercept are derived by well-known formulas of linear regression analysis. It is not actually necessary to produce the graphs of the lines in this method, because the estimates of n and log K are not read from graphs but are obtained from linear regression formulas. However, graphs of the stimulus response data are helpful in identifying the upper limb of the response curves and in choosing R_{max}.

An interesting consequence of Aylward's method [11] is that when the light stimuli increase in equal log steps, the linear regression formulas for n and log K condense to tractable formulas. This enables one to estimate the parameters of the Naka-Rushton equation without recourse to a computer or without the necessity of performing a regression analysis calculation on every data set.

First, denote by H the lowest luminance stimulus and let J be the factor by which luminance increases. This means that the log luminance of the ith stimulus will be log $H + (i - 1)$ log J. Let R_i denote the response to the ith stimulus. Estimate R_{max} as previously discussed by making it larger than the greatest response remaining after the upper limb of the response curve has been discarded.

Next, form the transformed responses $y_i = \log [(R_{max}/R_i) - 1]$ and the sums $S = [(m + 1)/2] \Sigma\ y_i$ and $T = \Sigma\ iy_i$ where m is the total number of stimuli. Then we have the following:

$$n = 12(S - T)/[m(m^2 - 1) \log J], \text{ and} \tag{4}$$

$$\log K = \log H + [(m - 1)/2] \log J + (1/3)[1 - (S/T)] \log J . \tag{5}$$

Inspection of these formulas shows that log H does not appear in Formula 4, which means that n is mathematically independent of the baseline stimulus. When Equation 1 is plotted in semilogarithmic coordinates as usual, the function is a sigmoid curve that is symmetric about the point (log $K, \frac{1}{2}R_{max}$), with the parameter n describing the shape of the curve rather than its location.

Formula 5 is a sum of three terms, the first two of which add up to the midpoint of the range of stimuli. The third term specifies how far from the midpoint one finds log K

Currently, flash stimuli cannot always be presented in equal log increments, so Formulas 4 and 5 are of limited practical utility. The problems attendant with the choice of R_{max} as discussed above must also be dealt with. Curiously, we have not encountered formulas analogous to 4 or 5 for the Hill equation or for any other application of the logistic equation, of which Equation 1 is a special case.

Curve-fitting method C used the nonlinear curve fitting program Systat. This program fits the Naka-Rushton equation to the initial portion of the luminance-response function. This function was truncated at $-0.7 \log \mathrm{cd}$ second$/\mathrm{m}^2$, near the point where the second limb begins [10].

Results

The data points in Fig. 1 present the luminance-response function obtained from subject 14. The three lines represent the solutions for the Naka-Rushton equation derived by means of the three curve fitting methods. In this case, all three methods successfully avoid data points comprising the second limb. As a result, the three fits provide similar functions.

To compare the parameters derived from the three curve-fitting methods more carefully, the values obtained from one method were plotted against those obtained from another method. A one-sided sign test was then used to compare the pairs of values obtained from each subject [14]. Figure 2 presents the derived R_{max} values. Method A consistently yielded greater values of R_{max} than did method B (Fig. 2a; $p < 0.01$) or method C (Fig. 2c; $p < 0.05$). The magnitude of the difference between the estimates was larger at higher values of R_{max}. In addition, method C gave greater values than did method B (Fig. 2b; $p < 0.05$), although the values of the actual discrepancies were seldom large.

Figure 3 compares the derived values of n. Method A consistently gave smaller values than did method B (Fig. 3a $p < 0.001$). Values obtained in

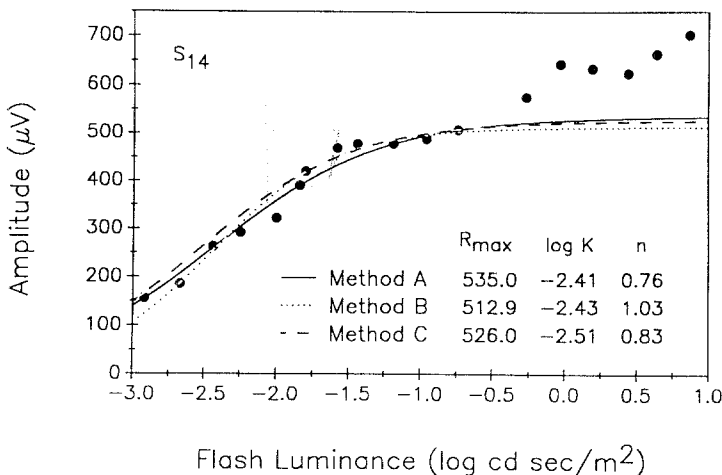|	R_{max}	log K	n
—— Method A	535.0	−2.41	0.76
······ Method B	512.9	−2.43	1.03
− − Method C	526.0	−2.51	0.83

Fig. 1. Luminance-response function obtained from normal subject 14. The three lines represent fittings to the Naka-Rushton equation derived with curve-fitting methods A through C.
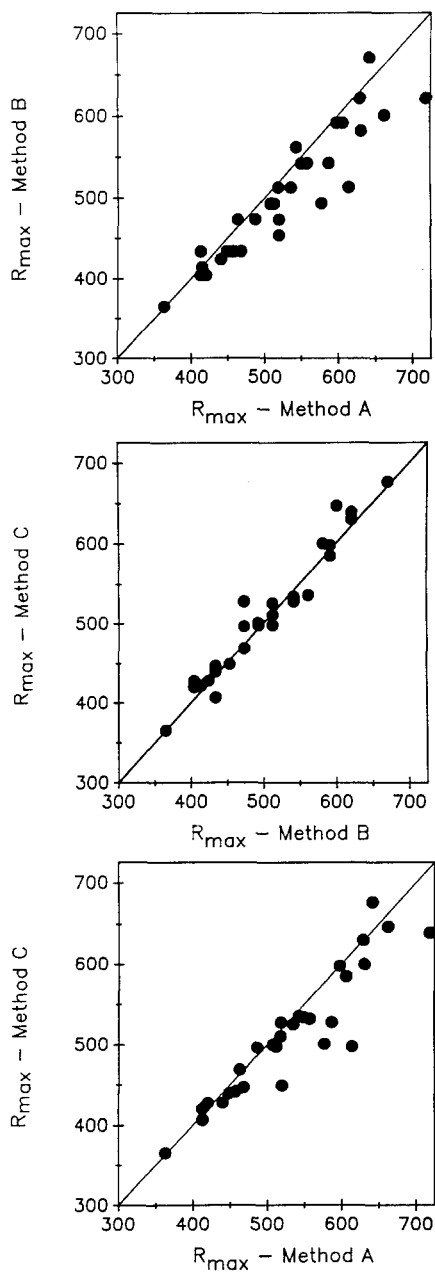
Fig. 2. Comparison of R_{max} values derived by methods A, B and C. Each panel compares the values derived from two methods. Each data point represents a separate subject. (a) Method B plotted against method A; (b) method C plotted against method B; (c) method C plotted against method A.
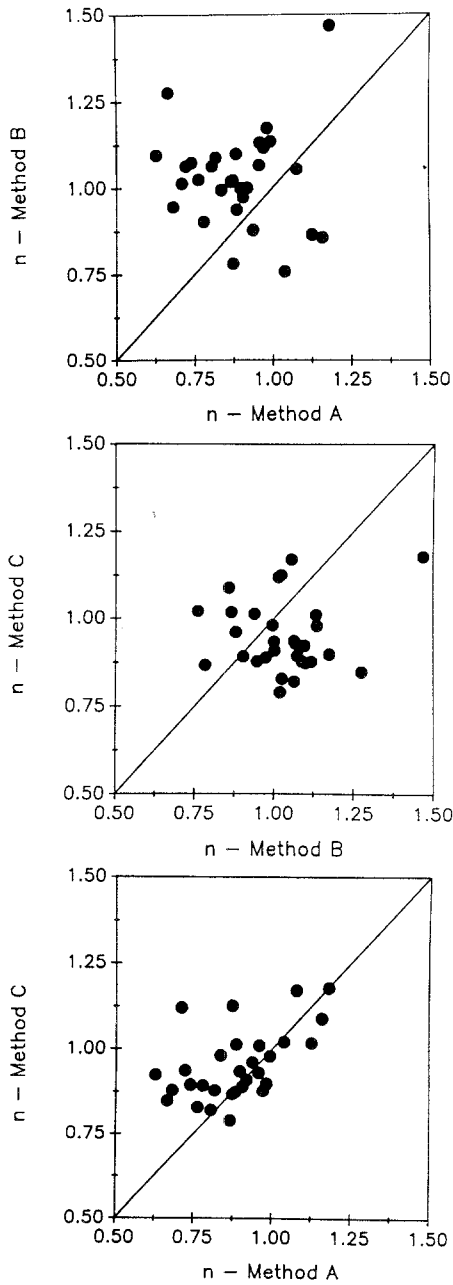
Fig. 3. Comparison of *n* values derived by methods A, B and C. Each panel compares the values derived from two methods. Each data point represents a separate subject. (a) Method B plotted against method A; method C plotted against method B (c) method C plotted against method A.
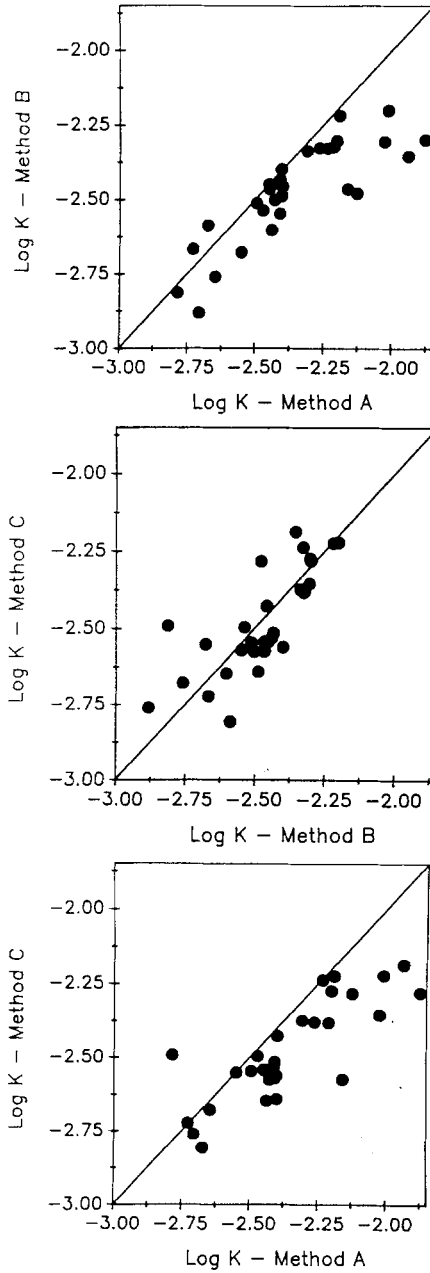
Fig. 4. Comparison of log *K* values derived by methods A, B and C. Each panel compares the values derived from two methods. Each data point represents a separate subject. (a) Method B plotted against method A; (b) method C plotted against method B; (c) method C plotted against method A.

methods A and C did not give consistently different values (Fig. 3B). Method B gave greater values for n than did method C (Fig. 3c; $p < 0.025$).

The values of log K are presented in Fig. 4. Method A consistently gave greater values than did methods B (Fig. 4a $p < 0.001$) and C (Fig. 4c; $p < 0.001$). The magnitude of the differences appeared to increase with higher values of log K. The values obtained from methods B and C were not consistently different (Fig. 4b).

Discussion

These results indicate the presence of systematic differences in the parameter values derived by the different curve-fitting methods. These differences may be an important consideration when results are compared from studies that used different methods to fit the Naka-Rushton equation to the luminance-response function. For R_{max}, method A yielded the largest values. This is likely to be due to some solutions incorporating one or more points of the second limb. This possibility is consistent with the reduced values of n and increased values of log K derived by method A.

With method B, three difficulties can be identified that impact on the initial step of choosing R_{max}. First for a noisy data set, identification of the upper limb of the stimulus response curve may be difficult. Having graphs of the data in addition to tables to inspect may enable easier identification of the upper limb. A second difficulty arises if R_{max} is taken as a fixed percentage greater than the actual maximum response (R_{peak}). After responses from the upper limb are rejected, the transformed value of R_{peak} will be the same in any data set to which the method is applied. In our calculation, the transformed value of R_{peak} was always -2, because we chose it to be 1% less than R_{max} after discarding the upper limb. Having this fixed value in the transformed data tends to steepen the regression line through the data, thereby raising the value of n. This effect may account for the tendency for method B to give greater values of n than did either method A or C. In his article, Aylward did not specify a fixed method for choosing R_{max} [11]. In this study, 1% was chosen to be consistent for the treatment of all data sets after trials with other percentages. A third weakness of method B is that the linearizing transformation depends on R_{max}. As a result, subsequent calculations for n and log K are sensitive to errors in the choice of R_{max}. The extent to which varying the initial R_{max} value will affect the derived values of n and log K is shown in Fig. 5a and 5b, respectively. For this illustration we used the data set published by Aylward [11], because the data are relatively noisefree and the second rising limb of the luminance-response function is easy to identify. The derived values of both n and K vary considerably as R_{max} changes. For example, a 3% change in R_{max} resulted in an 11% change in n (Fig. 5a) and a 7.7% change in log K (Fig. 5b). Such dependence on the choice of R_{max} may be
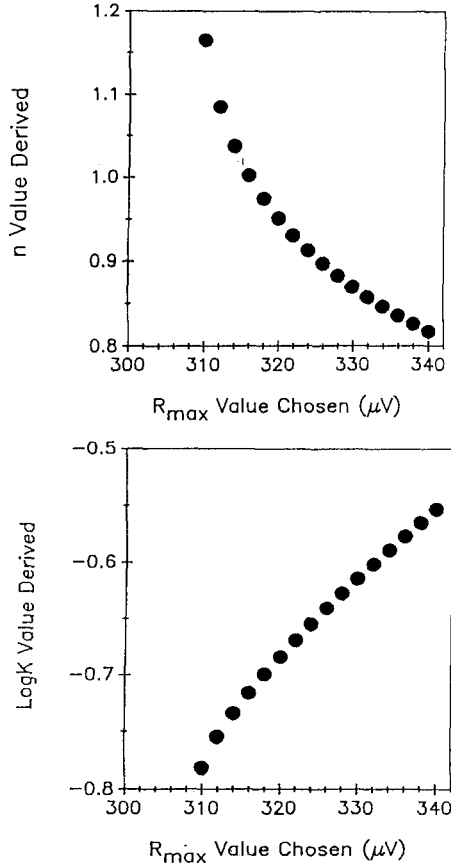
Fig. 5. Dependence of values of *n* (a) or log *K* on the initial value chosen for R_{max} by means of method B applied to the data set analyzed by Aylward [11].

exacerbated by noisy data, particularly where a single high amplitude point is present.

Each of the methods used in the present study solved for the value of *n* as well as for R_{max} and log *K*. The primary advantage of this approach is that the value of *n* may be used as an indicator of retinal homogeneity [2]. If a disease process has a uniform effect on the retina, then *n* values are expected to remain near 1.0; if a disease process increases log *K* values for some retinal areas but not others, then the value of *n* is expected to decrease [2]. In this light, setting the value of *n* at a constant value (typically 1.0) will limit the value of the analysis applied to patients with retinal disorders that result in retinal inhomogeneity. In addition, while the value of *n* averages near 1.0 for the normal dark-adapted luminance-response function [2], the corresponding value for the light-adapted cone ERG luminance-response function is above 1.5 [12, 15]. As a result, setting *n* to a value of 1.0 would lead to an inaccurate description of the cone ERG luminance-response

function. Therefore, although a number of studies have set the value of n to 1.0 [1, 3, 16, 17], we solved for this parameter in parallel with R_{max} and log K.

Other methods than those used in the present study have been developed to analyze the luminance-response function of the ERG. For example, Hood & Birch [17] described an approach to derive P3 and then P2, the primary components that decrease the ERG b-wave [18]. Although this approach has certain advantages over those used in the present study, it is not in common use. Until this changes, most analyses will employ a method similar to one used here. The possibility of consistent differences in estimates of the Naka-Rushton parameters obtained by various approaches may be an important factor when considering values published in the literature.

Acknowledgments

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