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Abnormalities of the Retinal Cone System in Retinitis Pigmentosa

DONALD C. HOOD,*† DAVID G. BIRCH‡

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Patients with retinitis pigmentosa (RP) show delayed inner retinal responses as measured by the cone ERG response to a 30 Hz stimulus. To determine the extent to which this delay results from abnormalities of cone phototransduction, cone ERGs to single flashes were obtained from 21 patients with RP and a model of cone phototransduction was fitted to the leading edge of the *a*-waves of these ERGs. Nearly all patients showed an abnormally low sensitivity of cone phototransduction consistent with a reduction in the amplification of transduction. This abnormality can account for part of the delayed 30 Hz response. Analysis of post-receptoral potentials indicated that RP also slows the responses of the inner retina. A combination of these two factors, a sensitivity change at the receptor and a delay in the response of the inner retina, produces the delayed response of the cone flicker ERG in patients with RP. Copyright © 1996 Elsevier Science Ltd.

Retinal disease Retinitis pigmentosa Cone receptor Electroretinogram Human

INTRODUCTION

The response of the inner retina as measured by the *b*-wave of the cone ERG is delayed in patients with retinitis pigmentosa (RP) [(e.g. Berson *et al.*, 1969a; Berson & Kanters, 1970; Massof *et al.*, 1986); see also Berson (1993) for a review]. Some have suggested that the cone ERG abnormalities are due, at least in part, to cone photoreceptor abnormalities, specifically to a decrease in quantal absorption by the cone outer segments (e.g. Sandberg *et al.*, 1981; Gouras & MacKay, 1989; Berson, 1993). This hypothesis is based upon abnormalities of the cone *a*-wave combined with anatomical studies showing that the cone outer segments are shortened at advanced stages of the disease (Kolb & Gouras, 1974; Szamier & Berson, 1977; Szamier *et al.*, 1979; Bunt Milam *et al.*, 1983; Flannery *et al.*, 1989; Li *et al.*, 1994). Other ERG results (e.g. Berson *et al.*, 1969b; Massof *et al.*, 1986; Seiple *et al.*, 1986; Miller & Sandberg, 1991; Falsini *et al.*, 1994), as well as behavioral data (e.g. Greenstein & Hood, 1986, 1992; Greenstein *et al.*, 1987; Tyler *et al.*, 1984; Massof *et al.*, 1988; Alexander *et al.*, 1991; Dagnelie & Massof, 1993a, b), are more difficult to reconcile with this simple cone receptor defect.

Specific hypotheses about human cone receptor activity can be tested by measuring the cone *a*-wave of the ERG (Hood & Birch, 1993a, 1995). There is reasonably strong evidence that the first 10 msec or so of the cone *a*-wave is the sum of the responses of the cone photoreceptors. The leading edge of the cone *a*-wave has properties similar to those of the cone receptor component of the monkey ERG (cf. Hood & Birch, 1993a, 1995; Sieving, 1993; Bush & Sieving, 1994). Further, it can be fitted with models (Hood & Birch, 1993a, 1995) that are similar to the models fitted to responses from single cones (e.g. Baylor, Hodgkin & Lamb, 1974; Schnapf *et al.*, 1990; Pugh & Lamb, 1993). Together these findings open the possibility of assessing phototransduction in human cones affected by retinal disease.

Recently, a model of cone phototransduction was fitted to the *a*-waves of five patients with RP (Hood & Birch, 1995). All five were found to have abnormal phototransduction. Here we fit this model to data from a larger group of patients. In addition, we ask whether changes in the cone photoreceptor parameters can account for the changes in the timing of the response of the inner retina. Preliminary versions of these findings were reported at meetings of the OSA (*Vision Science and Its Applications*, 1995), the *International Congress of Eye Research* (*Satellite Symposium on Retinal Degeneration*, Jerusalem, 1994), and ARVO (1995).

METHODS

Subjects

The subjects were placed into two groups based upon the adapting field used to isolate the cone response. After

*Department of Psychology, Columbia University, 406 Schermerhorn Hall, New York, NY 10027, U.S.A.
†To whom all correspondence should be addressed [*Email* don@psych.columbia.edu].
‡Retina Foundation of the Southwest, Dallas, Texas, U.S.A.

1 March 1994 a 3.4 log td field was used to isolate the cones as described below. Before this date, a lower adapting field (2.6 or 2.9 log td) was used. Subjects tested before 1 March 1994 will be referred to as Group A and those tested after this date as Group B. The results of these two groups are presented separately, although there were no material differences between their results.

Group A. Thirteen patients ranging in age from 11 to 47 yr (mean age = 30.2 yr) were in this group. They were classified as: eight adRP, three simplex, one X-linked, and one recessive. Six of the patients with adRP had a rhodopsin mutation [pro23his (three); leu46arg (two); and splice-17y (one)]. Four of these patients participated in the earlier study (Hood & Birch, 1995). Also included in this group were eight controls ranging in age from 33 to 51 yr (mean age = 42.5 yr) with normal color vision, normal full-field ERGs and normal ophthalmological examinations.

Group B. Eight patients ranging in age from 10 to 45 yr (mean age = 27.3 yr) and six normal controls ranging in age from 38 to 57 yr (mean age = 46.7 yr) were in this group. The patients were classified as: three adRP, three simplex, and two recessive.

All patients had been diagnosed by ophthalmologists specializing in retinal disease. The tenets of the *Declaration of Helsinki* were followed and all subjects gave written informed consent after a full explanation of the procedures was given.

Recording techniques

The methods used for obtaining full-field ERGs were relatively standard (Birch & Fish, 1987). One eye was dilated (1% cyclopentolate hydrochloride and 2.5% phenylephrine hydrochloride) and dark-adapted. Responses were obtained from the anesthetized cornea with a bipolar contact lens electrode with matched gold electrodes (Doran Instruments Inc., Littleton, MA). Signals were amplified (factor of 10,000; 3 dB down at 2 and 10,000 Hz) and averaged as described below.

Stimulation

All stimuli were presented in a Ganzfeld system. Standard protocol responses were obtained with a Grass photostimulator and high intensity responses were obtained with a light source consisting of a power supply (Novatron Inc., Dallas, TX) that drives a circular xenon gas flash tube within a flash head (Novatron series 2150). When set to 800 W/sec, this unit produces flashes in which 90% of the energy is within 1.3 msec. Three spectral flashes were used in this study: "white" flashes (spectrally unfiltered); short-wavelength ("blue") flashes (Wratten 47B); and long-wavelength ("red") flashes (Wratten 26). Retinal illuminance was determined by measuring the luminance of the Ganzfeld bowl and the diameter of the dilated pupil for each subject.

Dark-adapted Rod a-*waves*. The general procedures have been previously described (Hood & Birch, 1994). Briefly, following 45 min of dark-adaptation, responses were obtained in the dark to blue flashes. At least four flash intensities were used ranging up to 4.4 log scot td-sec. From three to ten responses were computer averaged. The small cone contribution was removed by computer subtracting responses to the photopically matched red flashes obtained against the adapting field used to isolate the cone response. Four of the normal subjects took part in a different study (Hood & Birch, 1995) and rod *a*-waves are not available for them.

Light-adapted Cone a-*wave*. Following the dark-adapted series described above, subjects in Group A were adapted to a 2.6 or 2.9 log td "white" field and subjects in Group B to a 3.4 log td field. After 5 min of adaptation, responses were obtained to the red flashes from 2.2 or 2.5 to 4.3 log td-sec in approximately 0.3 log unit steps. Following presentation of the red flashes, blue flashes in the presence of the background were presented to assess the isolation of the cones. In three patients (the two with the leu46arg mutation and the xlRP), there was no sign of rod activity and cone ERGs were obtained in the dark-adapted eye with white flashes to extend the range of flash intensities available. For each flash energy, 6–18 responses were averaged.

Cone Isolation and the Choice of Adapting Field Intensities. In earlier work (Hood & Birch, 1993a), we determined that the leading edge of the cone *a*-wave was unaffected by adapting fields below about 2.6 log td. The choice of the adapting field intensity involves a tradeoff between adaptation of the cone *a*-wave and suppression of the rod contribution. For Group A, the 2.9 log td field changed the *a*-wave sensitivity on average by <0.08 log unit, but the rods contributed to the response amplitude for the highest two red flashes (4.0 and 4.3 log td-sec) as indicated by the responses to the blue flashes. For this group, the responses to the two highest, red flash intensities were not included in the fitting. For Group B, the 3.4 log td field changed sensitivity by <0.2 log unit, and there was no sign of rod involvement, even with the most intense flashes. The change in protocol between Groups A and B represents a decision to use a background that adapts the *a*-wave slightly (<0.2 log unit), but that eliminates the rod contribution.

30 Hz Flicker. In all patients, responses were obtained to a 30 Hz, 1.3 log td-sec light and the implicit time of the primary positive component was measured. In addition, for three normal observers (ages 35, 51, and 52 yr) 30 Hz flicker ERGs were obtained to intensities ranging from −0.3 to 1.9 log td-sec.

Theoretical analysis

The Rod Model and the a-*wave*. The leading edge of the rod *a*-wave is the sum of the responses of individual rod outer segments (Hood & Birch, 1990a, b) and a model of the activation phase of rod phototransduction (Lamb & Pugh, 1992) describes its shape (Cideciyan & Jacobson, 1993; Hood & Birch, 1993b, 1994; Breton *et al.*, 1994). In particular, the leading edges of the rod *a*-waves are described by

$$P3(I,t) = \{1 - \exp[-I \cdot S \cdot (t - t_d)^2]\} \cdot Rm_{P3} \text{ for } t > t_d \quad (1)$$

where the amplitude P3, named after Granit's receptoral component, is a function of flash energy I and time t after the occurrence of a brief, essentially instantaneous, flash. S is a sensitivity parameter that scales I (flash energy); Rm_{P3} is the maximum amplitude; and t_d is a brief delay.

Rod *a*-waves were fitted after setting t_d to 3.2 msec, the mean of the best-fitting value for a group of normal subjects, by estimating two parameters [S (td-sec)$^{-1}$ sec^{-2}; Rm_{P3} (μV)]. Previous work has shown that patients' values of t_d do not differ from the normal values and that the value of t_d has a slight effect on the estimated value of S (Hood & Birch, 1994). Thus slightly less variable estimates of S can be obtained by fixing t_d. The methods used are described in Hood & Birch (1994).

Biophysical Bases of the Model. Following the isomerization of a molecule of rhodopsin, there is a cascade of events leading up to the closing of the cGMP-activated sodium channels. Equation (1) is based upon Lamb and Pugh's model of these forward going events (Lamb & Pugh, 1992; Pugh & Lamb, 1993). According to this model of the activation phase of rod transduction, the photocurrent of a rod following a brief flash that isomerizes ϕ rhodopsin molecules is

$$r(\phi, t) \cong \left\{1 - \exp\left[-\frac{1}{2}\phi \cdot A \cdot (t - t_{eff})^2\right]\right\} r_{max} \text{ for } t > t_{eff} \quad (2)$$

where A is an amplification constant in (isomerizations)$^{-1}$ sec^{-2}; r_{max} is the saturating photocurrent; and t_{eff} is a brief delay. Some of the very brief reactions in the activation cascade (e.g. the conversion to the activated forms of rhodopsin, the G-protein, and PDE, and the closure of the channels) are incorporated in the parameter t_{eff}. The amplification constant A comes from the sequential production of the activated forms of the G-protein and PDE, the hydrolysis of cCMP, and the relationship of cGMP to the closure of the channels. Equation (2) does not take into consideration the backward or deactivation steps of transduction and, thus, only predicts the leading edge of the rod responses. In primates, it describes the response to about 100 msec (Lamb & Pugh, 1992; Kraft *et al.*, 1993; Pugh & Lamb, 1993), far longer than the times used in the *a*-wave analysis. [See Lamb and Pugh (1992), Pugh and Lamb (1993), and Breton *et al.* (1994) for more details.]

Hypotheses about the defects at the receptors can be phrased in terms of the parameters of Eqn (2) and can be tested by estimating the parameters of Eqn (1) fitted to the leading edge of the *a*-wave [see Hood and Birch (1994) for a discussion].

The Model of the Cone a-*wave.* For our purposes, the rod model has a shortcoming. It is a model of rod photocurrent and we seek to describe cone voltage measures. According to Pugh and Lamb (1993), Eqn (2) should describe the activation phase of cone transduction, but it does not describe the cone voltage measures because the capacitance of the extensive cone outer segment membrane modifies these measures. In fact, Hood and Birch (1995) showed that Eqn (1) does not provide a good fit to the leading edge of the cone *a*-wave. Following the suggestion of Pugh and Lamb (1993), Hood and Birch (1995) modified the rod model by adding an exponential filter with a time constant of t to account for the capacitance effects of the cone outer segment membrane. (In particular, the output of the transduction process [P3 as given by Eqn (1)] is convolved with $\exp[-(t/\tau)]$.) The meaning of S and Rm_{P3} are the same in both the rod and cone models.

In this study, the leading edge of the cone *a*-waves of all subjects was fitted by setting $t_d = 1.7$ msec and $\tau = 1.8$ msec, the average parameter values for a group of normals (Hood & Birch, 1995), and estimating the values of S and Rm_{P3} for best fit. Both τ and t_d influence to some extent the estimate of S. Thus, fixing these parameters has the advantages of decreasing the variability in the estimate of S (Hood & Birch, 1995). The fit to the patients' *a*-waves could not be improved by changing these parameters. In particular, the *a*-waves of the patients with abnormal S values could not be fitted by fixing the value of S at the normal value and varying τ and/or t_d. The methods used are described in Hood and Birch (1995).

EXPERIMENT 1: ASSESSMENT OF RECEPTOR FUNCTION

Rod a-*waves*

Figure 1 shows rod *a*-waves for three subjects from Group B: one normal subject (A) and two patients (B and C). The solid curves are the rod ERGs elicited by flashes that ranged from 3.5 to 4.4 log td-sec. The dashed curve shows the fit of the model [Eqn (1)] obtained by estimating the two parameters, S and Rm_{P3}, as described above. The parameter values of best fit are given in the figure caption.

Each data point in Fig. 2(A) is the value of the log maximum rod *a*-wave amplitude [Rm_{P3}(rod)] plotted against the log of the rod sensitivity parameter [S(rod)]. The parameters are shown for the normal subjects as the open symbols and for the patients as the solid symbols (○, ● Group A; □, ■ Group B). The solid lines are the means of the parameters for the normal subjects and the dashed lines show the lower range of these values. As previously observed, the patients have a wide range of log S(rod) values, including near-normal values, and all patients show a diminished Rm_{P3}(rod) (Hood & Birch, 1994; Shady *et al.*, 1995).

Cone a-*waves*

The solid curves in Fig. 3 are the first 45 msec of the computer averaged cone ERG. The dashed curve shows the fit of the cone model obtained by estimating the two parameters, S and Rm_{P3}, as described above. All *a*-waves were fitted up to 10.8 msec, but the theoretical curves are shown for the first 20 msec. The model fits the data well. The parameters of best fit are given in the figure caption.

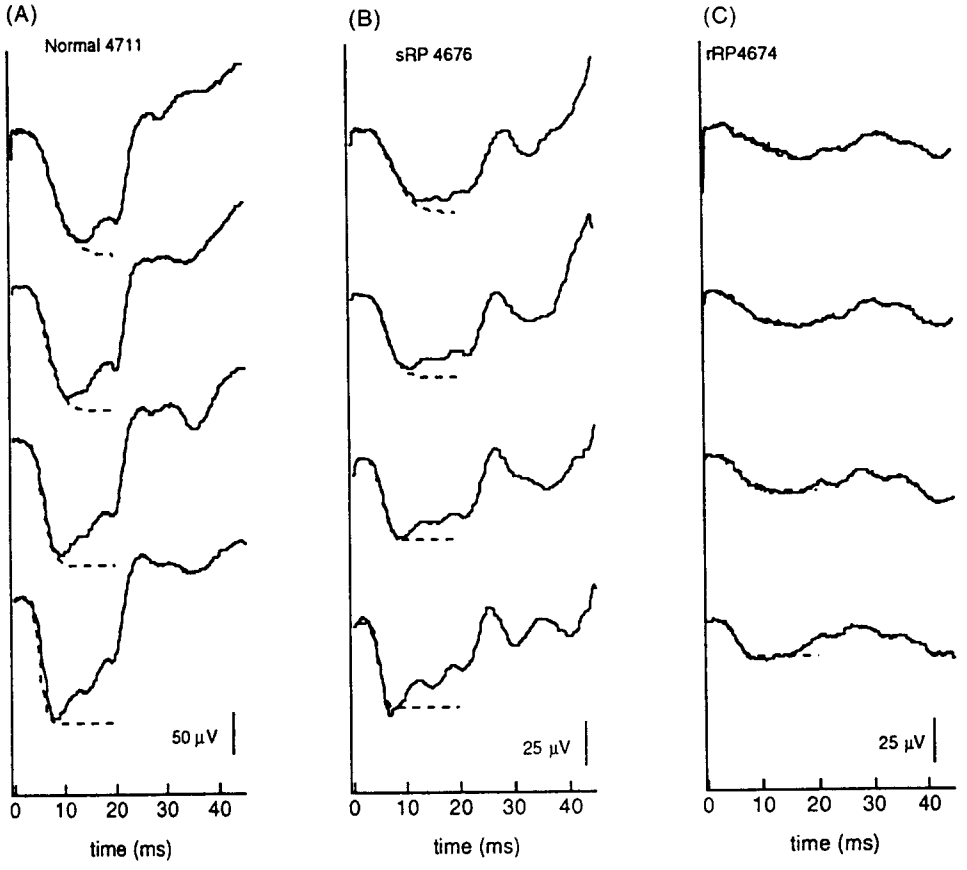

FIGURE 1. The records (——) are the rod ERGs from a normal subject (A), and two patients (B, C) with RP. The flashes ranged from 3.5 to 4.4 log scot td-sec in approximately 0.3 log unit steps. The (– – –) curves are the predictions from the model [Eqn (1)] and the parameters [log S (td-sec)$^{-1}$ sec^{-2}; Rm_{P3} (μV)] of best fit were [0.90; −160], [0.83; −22], and [0.93; −52] for the normal subject and two patients, respectively. Eqn (1) was fitted to the leading edge of the a-wave, but is shown for the first 20 msec.

Both patients had smaller values of Rm_{P3}(cone), as expected, since the disease leads to a loss of both rod and cone receptors. Both also had a value of S(cone) that was below the mean normal value.

Figure 2(B) shows the values of log S(cone) and log Rm_{P3}(cone) for all subjects. The normal subjects' parameters are shown as the open symbols (○, Group A parameters; □, Group B parameters). As expected from

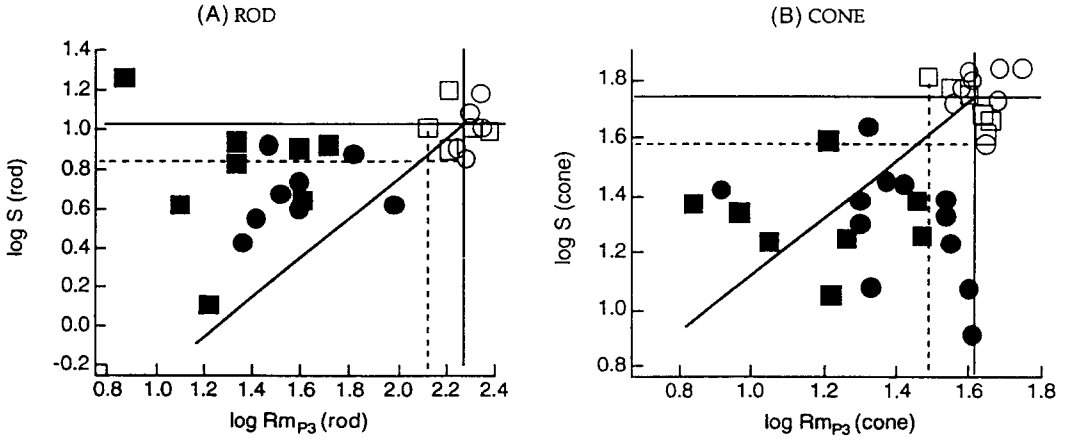

FIGURE 2. (A) Each data point shows the log S and log Rm_{P3} parameters for the best fit of the rod a-waves from a single subject. The open symbols are the values for the normal subjects and the solid symbols are for the patients; Groups A and B are shown as the circles and squares, respectively. The solid horizontal and vertical lines indicate the mean normal values and the dashed lines show the lower limit of the range. The diagonal line has a slope of 1 and represents the loci of equal changes in the log values of the parameters. (B) The parameter values for the cone a-waves shown as in (A).

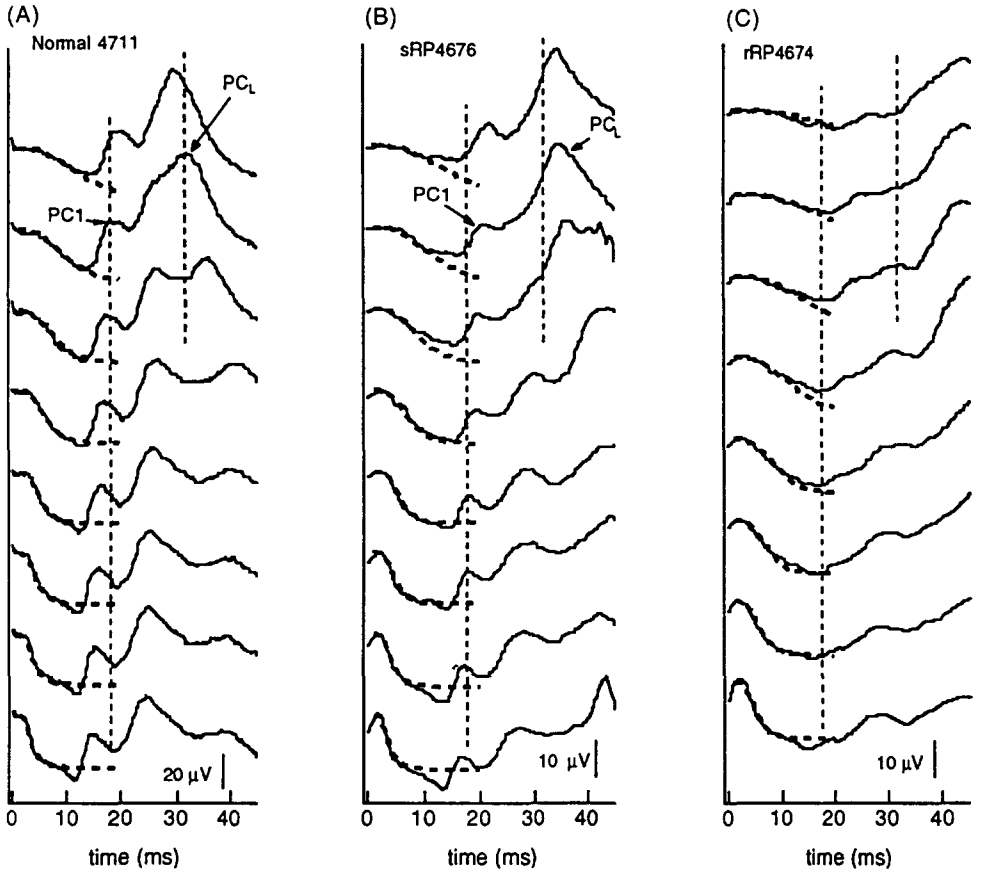

FIGURE 3. The records (——) are the cone ERGs from a normal subject (A), and the same two patients (B, C) with RP as in Fig. 1. The red flashes ranged from 2.1 to 4.3 log td-sec in approximately 0.3 log unit steps. The dashed curves are the predictions from the model and the parameters [log S ((td-sec)$^{-1}$ sec^{-2}); Rm_{P3} (μV)] of best fit were [1.81; −31], [1.05; −17], and [1.59; −16] for the normal subject and two patients, respectively. The model was fitted to the first 10.8 msec of the leading edge of the a-wave, but is shown for the first 20 msec. Two post-receptoral components (PC1 and PC_L) are also labeled. The vertical lines show the latency of these components for the normal subject in (A) for the flash energy indicated by the arrow.

previous work (Hood & Birch, 1993a), the means of the parameter values for Group B (the 3.4 log td field) are slightly smaller (by about 0.05 log unit) than the values for Group A (the lower field intensity). This difference is minor relative to the spread of the normal values and the size of the effects of disease. The solid lines are the means of the parameters for all the normal subjects and the dashed lines show the range of the normal values.

Fifteen of the 21 patients had values of Rm_{P3}(cone) that were outside the 95% confidence limits for the normals; and all but two patients had log S(cone) values that fell outside these limits. [The patients whose records are shown Fig. 3 had the highest (B) and lowest (C) values of log S(cone) in Group B (□, ■ in Fig. 2).] This essentially confirms, for a much larger group of patients, the finding of Hood and Birch (1995) that patients with RP show large S(cone) changes.

There are two striking differences between the rod and cone parameters in Fig. 2. First, nearly all patients had rods with greater losses in log Rm_{P3} than in log S, while for the cones the tendency is the reverse. (The diagonal line in both figures is the locus of equal decreases in both parameters.) Second, many patients have a log S(rod) value that is near normal (Cideciyan & Jacobson, 1993; Breton *et al.*, 1994; Hood & Birch, 1994; Shady *et al.*, 1995), while the value of log S(cone) is nearly always abnormal. Figure 4 shows a comparison of the parameters for the rods and cones; the diagonal line is the locus of equal decreases in both parameters. The change in log Rm_{P3}(rod) is always greater than the change in log Rm_{P3}(cone) [Fig. 4(A)], whereas in the overwhelming majority of the patients, the change in log S(cone) was greater than the change in log S(rod) [Fig. 4(B)].

DISCUSSION OF RECEPTOR FUNCTION

With disease progression, it is well known that both rod and cone receptors undergo degeneration in patients with RP. The most common explanation for a change in the maximum amplitude of the rod and cone a-waves (Rm_{P3}) is based upon the anatomical observation (Bunt-Milam *et al.*, 1983; Flannery *et al.*, 1989; Kolb & Gouras, 1974; Szamier & Berson, 1977; Szamier *et al.*, 1979; Li *et al.*, 1994) that many receptors are missing and others appear to have shortened outer segments. These morphological changes do not provide an explanation for the decrease in sensitivity (S) of the rods or cones. Shortened outer

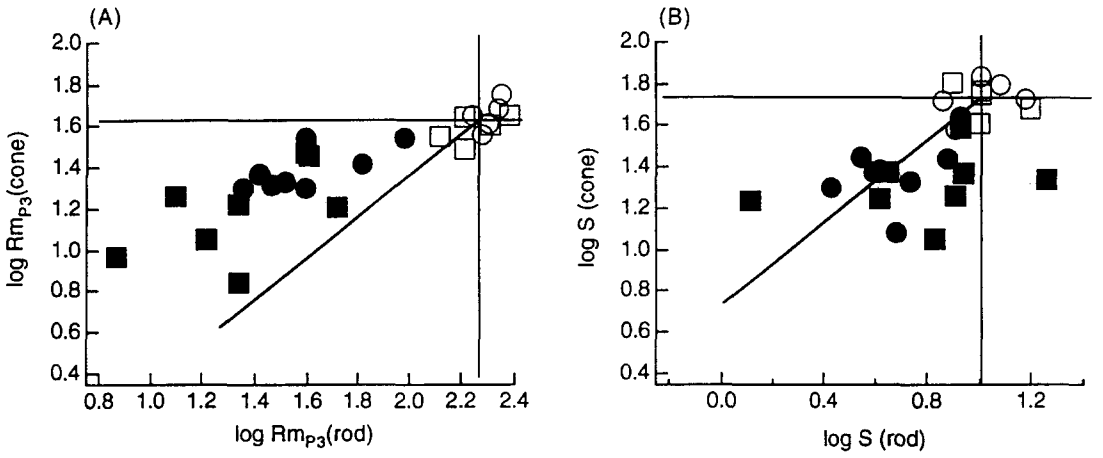

FIGURE 4. (A) Each data point shows the log Rm_{P3} values for the best fit of the cone and rod *a*-waves from a single subject. The open symbols are the values for the normal subjects and the solid symbols are for the patients; Groups A and B are shown as the circles and squares, respectively. The solid horizontal and vertical lines indicate the mean normal values. The diagonal line has a slope of 1 and represents the loci of equal changes in the log values of the parameters. (B) As in (A) but for the log *S* values.

segments or missing receptors will decrease Rm_{P3} but they should leave *S* normal (Breton *et al.*, 1994; Hood & Birch, 1994; Shady *et al.*, 1995).

The depressed values of *S*(cone) are consistent with an abnormal activation phase of most of the functioning cones in the eyes of patients with RP. It is useful to distinguish between two classes of mechanisms for these changes, those that alter the local quantal catch [a change in ϕ in Eqn (2) for any given intensity *I*] and those that alter one or more of the stages following isomerization [a change in *A* in Eqn (2)]. Histological studies have shown that the outer segments of the cones are disoriented with disorganized lamellae (Kolb & Gouras, 1974; Szamier *et al.*, 1979; Flannery *et al.*, 1989). These morphological changes in the cones could lead to a decrease in local quantal catch due to a change in wave guide properties of the outer segments (e.g. Birch & Sandberg, 1982) or to a decreased efficiency in the biochemical stages of transduction [a change in *A* in Eqn (2)]. In either case, it is likely that these morphological changes in the cones are secondary to degeneration in the rods. Consistent with this view, the values of log *S*(cone) are correlated ($r^2 = 0.66$) with the values of Rm_{P3}(rod) and this correlation is even higher than the correlation ($r^2 = 0.30$) between log *S*(rod) and log Rm_{P3} (rod). Although the results are consistent with degenerating rods somehow affecting the transduction of the cones, the magnitude of the changes in log *S* of the cones is still surprising. It is easy to understand the smaller changes in Rm_{P3} of the cones as compared to the rods (Fig. 4) based upon the greater loss of rod receptors seen morphologically. The greater range of log *S*(rod) values compared to log *S*(cone) values is also understandable on the grounds that the patients represent a variety of genetic variations of the disease and thus more than one factor may be contributing to rod degeneration. However, the larger changes in log *S* of the cones than in the rods (Fig. 4) are not as easily understood. One possible explanation is that the more rapid death of the rod receptor makes it less likely that a rod is functioning with a depressed sensitivity at any particular stage of disease progression.

In the next two sections, we consider whether the changes at the cone receptor can account for the changes in the response of the inner retina.

EXPERIMENT 2: DELAYS OF THE 30 HZ FLICKER RESPONSE

In the earliest stages of disease, patients with RP exhibit delays in the ERG response to a 30 Hz flickering light [(e.g. Berson *et al.*, 1969a, b; Berson & Kanters, 1970; Massof *et al.*, 1986); also see Berson (1993) for a review]. For the 21 RP patients in the present study, the implicit times for the responses to the 30 Hz stimulus (1.3 log td-sec) used in the clinical protocol ranged from 30.5 to 45.6 msec, compared to a mean normal value of 28.9 msec (Birch & Anderson, 1992); and all but one of the patients had an implicit time that was >2 SD above the mean. If the only effect of RP on the cone system is a decrease in sensitivity of the activation phase of transduction, as measured by the change in *S*(cone), then it should be possible to mimic the changes seen in patients by decreasing the intensity of the flicker stimulus in normal subjects.

The open symbols in Fig. 5(A) show the change in implicit time with change in the luminance of the white test flash for three normal subjects. The arrow shows the luminance of the 30 Hz flicker used in the clinical protocol. The large open square is the mean at that luminance for a group of normal subjects (Birch & Anderson, 1992). The solid symbols are the implicit times for the patients.

It is clear that a decrease in intensity increases the implicit times in the three normal observers. As the flash was decreased in intensity, the implicit time increased from $\leqslant$27 to 40 msec. All but one of the patients have implicit times within this range. Decreasing log *S* is effectively equivalent to decreasing the log of the

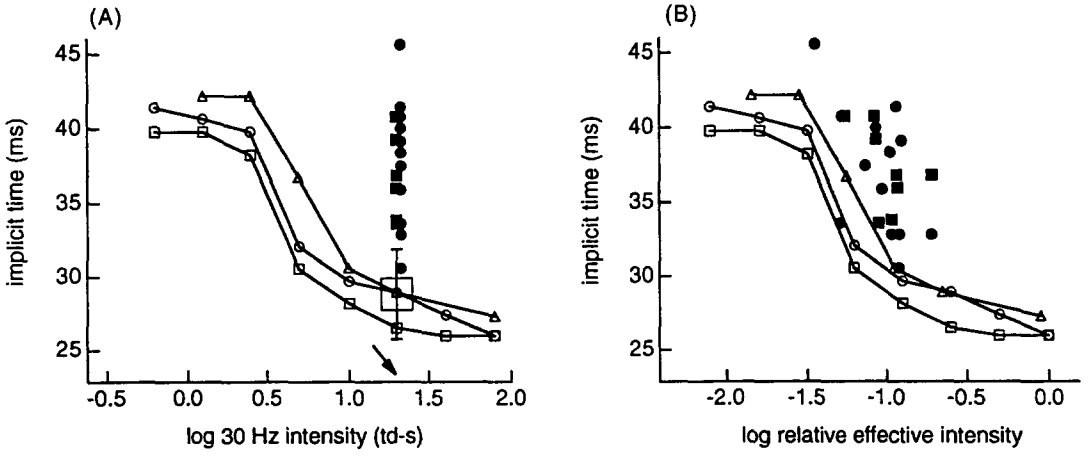

FIGURE 5. (A) The implicit time of the 30 Hz flicker response is shown as a function of flicker intensity for three normal subjects (open symbols). The solid symbols are the implicit times of the patients' 30 Hz responses plotted at the intensity (1.3 log td-sec) (see arrow) used in the standard clinical protocol. The large open square and accompanying bars show the mean and 2 SDs for a group of 50 normals. (B) As in (A) except that each patient's data point has been shifted horizontally by the difference between their log S(cone) value and the mean normal value. See the text for details.

intensity of the flickering light by the same amount. [The term $I \cdot S$ in Eqn (1) can be written as $\log I + \log S$.]. Thus, a decrease in $\log S$ would be expected to decrease the effective intensity of the stimulus and to increase the patients' implicit times. To test if the decrease in S could account for the increased implicit times, each patient's implicit time is plotted in Fig. 5(B) against the log of the flash intensity adjusted by the patient's individual change in $\log S$. Adjusting the intensity for the change in sensitivity brings the patients' values closer to the normal values. However, nearly all the patients values still fall above the normal values.

It appears that the change in $\log S$ can account for some, but not all, of the increase in implicit times to the 30 Hz stimulus. This finding is consistent with a number of studies that concluded that the effects of RP on the cone system cannot be mimicked by a decrease in the effectiveness of the light (e.g. Berson *et al.*, 1969b; Greenstein *et al.*, 1987; Tyler *et al.*, 1984; Greenstein & Hood, 1986, 1992; Massof *et al.*, 1986, 1988; Seiple *et al.*, 1986, 1993; Miller & Sandberg, 1991; Alexander *et al.*, 1991; Dagnelie & Massof, 1993a, b). Some factor(s) other than reduced receptor sensitivity must be contributing to the increase in the implicit time of the 30 Hz response. The analysis in the next section suggests that a change in the timing of the INL response is involved.

EXPERIMENT 3: POST-RECEPTORAL MEASURES OF THE CONE SYSTEM

The normal cone ERGs in Fig. 3 show a number of discernible positive peaks or bumps. Here we measured the implicit times of the first and last of these. The first positive bump, labeled PC1 for "positive component 1", appears to be what others have called either OP1 (e.g. Peachey *et al.*, 1991a; Kergoat & Lovasik, 1990; Murayama & Sieving, 1992) or OP2 (e.g. Lachapelle *et al.*, 1983). Its latency decreases with increased flash energy (e.g. Lachapelle *et al.*, 1983). The vertical dashed line near these waves in Fig. 3 marks 18 msec. The lower points in Fig. 6(A) show the implicit time of this wave as a function of flash energy for the six normal subjects in Group B. The agreement among subjects is good. The averages of these values are shown in Fig. 6(B) as the large open circles. As flash energy is increased, the implicit time decreases from about 20 to about 15 msec.

The solid symbols in Fig. 6(B) show the implicit times for the six patients in Group B whose records contained measurable PC1s. This potential is not discernible in the records of some patients (e.g. Sandberg *et al.*, 1981). In other patients, this post-synaptic potential is discernible but one cannot identify it with confidence [e.g. records in Fig. 3(C)]. For all but one of the six patients in Fig. 6, the implicit times of PC1 were elevated relative to normal. This can also be seen in Fig. 3 where the left vertical line in all panels marks 18 msec. [The analysis shown in Fig. 6 was not completed on the patients in Group A as different background intensities were used and the field intensity affects the implicit times and saliency of these potentials. However, an examination of their records indicates general agreement.]

To see if the change in log S(cone) could account for these delays, the implicit times are plotted in Fig. 6(C) against the log effective intensity by shifting them along the log intensity axis by the decrease in $\log S$. The change in $\log S$ cannot account for the delays. Figure 6(D) shows that these curves can be brought into line with a vertical shift. Here the data from the third panel have been shifted vertically to coincide with the data from the normals. It is as if these potentials are delayed by a constant amount at all flash energies by values ranging up to 3.2 msec. The open symbols in Fig. 7 show that the delays in the patients' 30 Hz implicit times (i.e. implicit time minus mean normal value) are correlated with the delays in PC1 (i.e. the shifts needed to bring the PC1 implicit times into line). The correlation ($r^2 = 0.62$) is reasonably good but

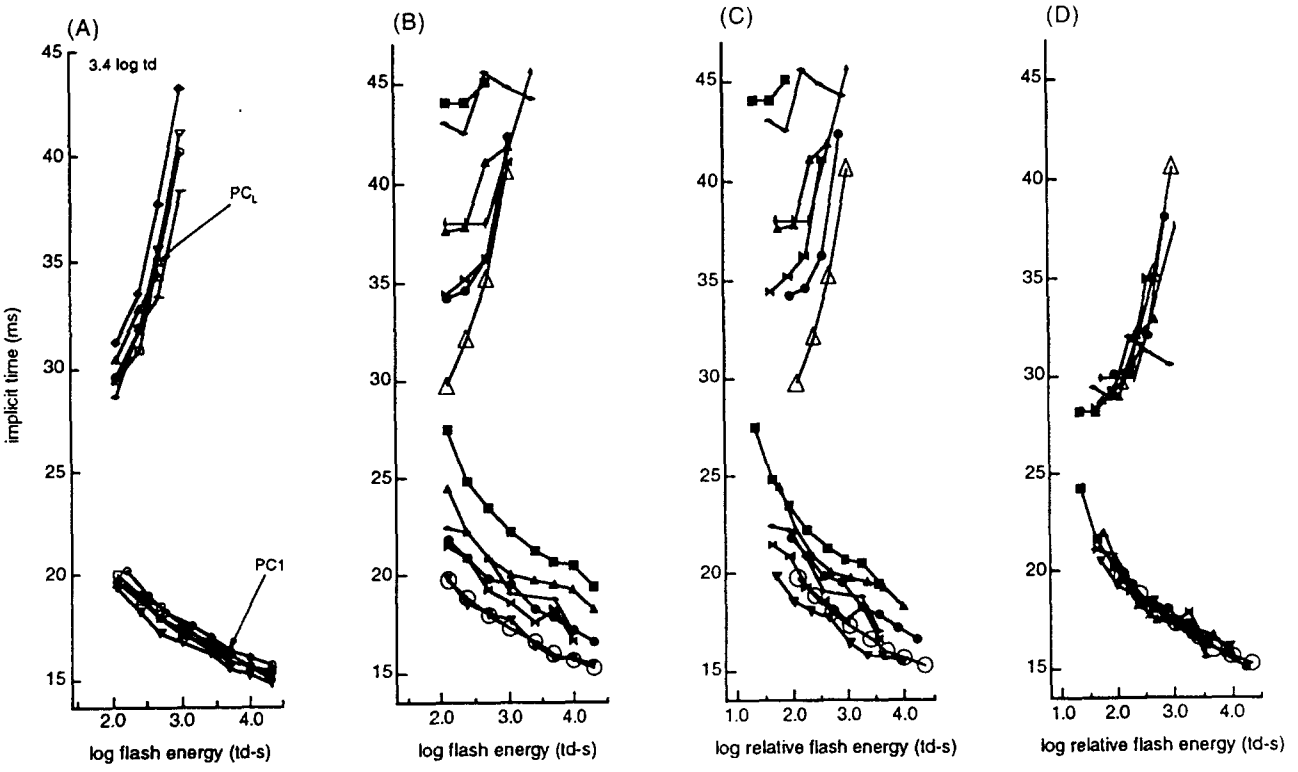

FIGURE 6. (A) The implicit time for the two post-receptoral components labeled in Fig. 3 is shown as a function of flash energy for the six normals in Group B. (B) The open symbols are the means of the normal values in (A); the solid symbols show the implicit times for seven RP patients. (C) Same as in (B) but each patient's data have been shifted horizontally by the difference between their log S(cone) value and the mean normal value. (D) Same as in (C) but each patient's data have been shifted vertically for best fit to the mean normal curve. See the text for details.

the delay in PC1 is considerably less than the delay in the implicit time.

The same conclusion results from an analysis of the potential labeled PC_L in Fig. 3. This potential has the characteristics of a potential identified as an off response by others (Nagata, 1963; Kojima & Zrenner, 1978; Walters *et al.*, 1981; Young, 1991; Alexander *et al.*, 1992; Sieving, 1993; Bush & Sieving, 1994) and probably corresponds to OP4 of Lachapelle *et al.* (1983). As the association of this potential with an off response remains controversial (Seiple & Holopigian, 1994), we follow Kojima and Zrenner (1978) in identifying it as the last (L) potential. Notice that its implicit time, unlike PC1, increases rather than decreases with increases in flash energy (Kojima & Zrenner, 1978). The implicit times of PC_L for the six normal subjects are shown in the upper part of Fig. 6(A). The implicit time varies from about 30 to 41 msec over the range of flash energies for which the potential can be measured. The rightmost vertical marker is set at 32 msec in Fig. 3. As can be seen in Figs 3(B) and 6(B), the implicit time of this component is elevated for the six patients in Group B in whose records this potential could be measured. (Five of these patients are the same ones for which PC1 could be measured. In two patients, only one of the two potentials could be measured with confidence.) As with PC1, correcting for the change in log S [Fig. 6(C)] cannot account for these changes, but, unlike with PC1, the correction moves the points in the wrong direction. A vertical shift of between 4.1 and 15.8 msec [Fig. 6(D)] does bring the patients' curves in line with the mean normal curve (△). The solid symbols in Fig. 7 show that

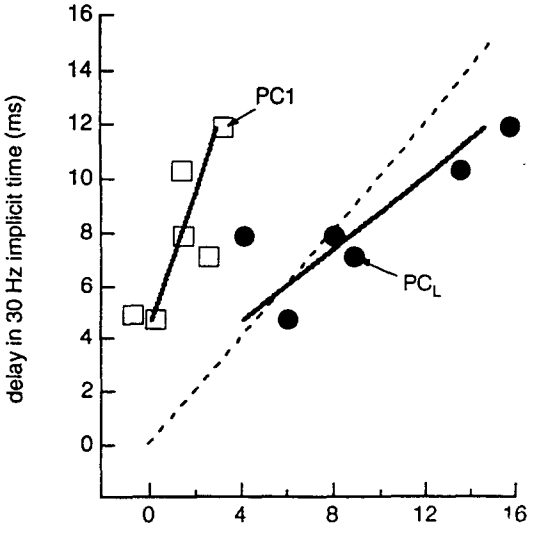

FIGURE 7. The delay in the patients' 30 Hz implicit times (patient's implicit time minus mean normal implicit time) are shown vs the delays in PC1 and PC_L [vertical shifts needed to bring the patient's data into line in Fig. 6(D)]. The solid lines are the best fitting straight lines and the dashed line is a line of slope 1.

the delays in the patients' 30 Hz implicit times are correlated with the delays in PC_L (i.e. the shift needed to bring the implicit times of PC_L into line). The correlation ($r^2 = 0.69$) is reasonably good and the magnitude of the delay in PC_L is close to the delay in the 30 Hz implicit time; the dashed line has a slope of 1.

GENERAL DISCUSSION

RP results in both a change in the sensitivity of the cone transduction process and a delay in the responses of the inner retina that cannot be accounted for by this sensitivity change. Together these two factors are responsible for the delay in the flicker cone ERGs. But, a complete understanding of how these two factors are involved must await a model of both the receptor and post-receptoral components of the cone ERG. The current model of the activation phase of transduction only predicts the early portion of the response at the cone outer segment. However, we can speculate about how the factors combine to delay the patients' 30 Hz responses. It is likely that the peak of the 30 Hz flicker response is dominated by PC_L in normal observers (Nagata, 1963; Birch & Sandberg, 1987). Thus, the delay in the patients' flicker ERGs is probably caused in large part by a delay in PC_L. Part of this delay is independent of the change in cone receptor sensitivity (log S) and, like the delay in the timing of PC1, is due to changes beyond the outer segment. Part of the delay in PCL is probably secondary to the change in receptor sensitivity (log S). A decrease in the receptor sensitivity in patients, or lowering the flicker intensity in normal subjects (Fig. 5), lowers the adaptation level. Lowering the level of adaptation is known to delay the cone inner retinal responses including PC_L (Kojima & Zrenner, 1978; Gouras & MacKay, 1989; Nagata, 1963; Peachey *et al.*, 1990, 1991a, b). Therefore, the changes in receptor sensitivity may affect the 30 Hz response primarily through its influence on the adaptation level.

The changes in timing of the different retinal components are also not simply affected by the disease process. The timing delays measured in the patients' cone ERGs are different for the different components (e.g. PC1 and PC_L in Fig. 6). This supports the conclusion of ERG studies that used sinusoidally flickering lights (Massof *et al.*, 1986; Seiple *et al.*, 1986, 1989). These studies concluded that the temporal abnormalities in the RP flicker ERG were not due to a reduced retinal quantal sensitivity, but required a slowing of the retinal response. They further concluded that this slowing was not a simple delay but was what Massof *et al.* (1986) referred to as a "smear-out" of the waveform in time, with later components being delayed relatively more than early waves. Both of the factors identified in the present study will affect the later components more than the earlier ones.

Because the ERG is a summed response of all the functioning cones, the foveal cones have a negligible effect on the responses recorded in the present study. However, studies designed to assess the temporal properties of the fovea have found evidence for a slowing of foveal cone function in patients with RP. Biersdorf (1981/1982) reported that foveal (central 4 deg) flicker ERGs were delayed in about one-third of his sample of RP patients. Based upon temporal contrast sensitivity measures, two psychophysical studies concluded that RP can slow the response of the foveal cone system (Tyler *et al.*, 1984; Dagnelie & Massof, 1993a, b). The Dagnelie and Massof study showed that the foveal cone system is altered early in the time course of the disease by a decrease in sensitivity followed by a timing change. While their findings agree well with ours, their explanation is different. They concluded that these timing changes involved the transduction process of the cone receptors, while we place much of the timing changes beyond the outer segment of the cone. Others have also concluded that RP affects post-receptoral sites (e.g. Greenstein & Hood, 1992; Cideciyan & Jacobson, 1993; Falsini *et al.*, 1994).

The cause(s) of the timing changes in the inner retina is still unknown. Like the changes in the sensitivity, S, of the cones, it is likely that the timing changes are secondary to rod degeneration. Birch and Sandberg (1987) reported a negative correlation between the cone implicit time and rod *b*-wave amplitude. That is, a patient's cone implicit time tended to be faster if rod *b*-wave amplitude was larger. It is unlikely that the source of the delay in the cone response of the inner retina is at the outer segment of the cones. However, we cannot rule out the cone receptor synapse as the locus, although the fact that the later waves are more delayed opens the possibility that more than one post-receptoral site may be involved.

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