

The Rod Photoreceptors in Retinopathy of Prematurity

An Electoretinographic Study

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Objective: To test the hypothesis that the more severe the acute phase retinopathy of prematurity (ROP) was in the preterm weeks, the more severely compromised is rod photoreceptor function after the ROP has resolved.

Methods: Electoretinographic (ERG) responses were recorded from 25 dark-adapted children (ages 2.5 months' postterm to 14 years) categorized by maximum, acute phase ROP (None to Very Severe). From the ERG a-wave "S," a sensitivity parameter for the rod photoreceptor response, and R_{mp3} , the saturated amplitude of the rod photoreceptor response were calculated using a model of the activation of rod phototransduction. The patients' results were compared with those of healthy controls (n=71).

Results: Among those in the None, Mild, Moderate, and Severe categories, both S and R_{mp3} varied significantly with severity of acute phase ROP. In the Very Severe category, ERG responses were too attenuated to calculate S and R_{mp3} .

Conclusions: The rod photoreceptors must be involved in ROP. The more severe the acute phase ROP, the more severe is the compromise of the processes involved in the activation of phototransduction in the rods.

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THE CLINICAL hallmark of retinopathy of prematurity (ROP) is abnormal retinal vasculature.¹ On the other side of the retina, the photoreceptors have no role in ROP according to conventional wisdom. Nevertheless, in 5 patients with a history of mild (stage 1 or stage 2) ROP that had resolved completely without any intervention, and in an additional 4 patients included in a report about refractive errors in ROP, there was electoretinographic (ERG) evidence of abnormal rod photoreceptor function.^{2,3} Additionally, elevations of scotopic visual thresholds indicate photoreceptor involvement in children with a history of resolved, mild ROP.^{4,5} In a rat model of ROP, structural and biochemical alterations in the rod outer segments have been documented.⁶ The rats had the same type of ERG abnormalities⁷ as the patients with ROP.^{2,3} These studies have led to the suggestion that photoreceptors are involved in the ROP disease process.⁶ After all, ROP has its onset at preterm ages,⁸ during which the rod photoreceptor outer segments elongate rap-

idly.^{9,10} As the outer segments grow, the needs for oxygen escalate to meet the demands for energy used in phototransduction processes, outer segment turnover, and the sodium pumps for the photoreceptors' circulating current.¹¹⁻¹⁶ Therefore, it is reasoned that as the preterm infants' photoreceptors demand more oxygen, the remainder of the retina becomes relatively hypoxic.^{2,3,6,17} Even in normal circumstances, the photoreceptors have just enough oxygen to maintain normal structure and function.^{15,18} Thus, if the oxygen needs are not met, the preterm infant's photoreceptors become damaged. According to this perspective, the more severe the photoreceptor involvement and retinal hypoxia, the more severe the ROP.

A small series of former preterm infants, categorized according to severity of acute phase ROP, is presented. Their rod photoreceptor function has been studied using contemporary ERG procedures and analyses. We tested the hypothesis that the more severe the acute phase ROP, the more severely compromised is rod photoreceptor function.

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PATIENTS AND METHODS

PATIENTS

The former preterm infants, who had been examined in the nursery for ROP, were recruited by mail. Excluded were those receiving ventilation at the time of the ROP examinations or on supplemental oxygen at the time of the ERG test. The patients (**Table 1**) are categorized (None, Mild, Moderate, Severe, and Very Severe) by maximum, acute phase ROP. The International Classification of ROP system was used to specify the severity and extent of acute ROP.^{1,19} Sixteen patients (Table 1) had standardized examinations^{1,19} in the newborn nursery by two of us (D.K.V. or R.A.P.), who were certified for the Supplemental Therapeutic Oxygen for Prethreshold Retinopathy of Prematurity study.²⁰ Nine infants (Table 1, patients 4, 5, 7, 13, 15, 16, 19-21) have been included in previous reports.^{2,3} Gestational age (Table 1) varied significantly in the ROP group ($F_{4,20}=7.4, P<.01$). Although many of the patients had mild neuromotor handicaps, all patients have been in good general health, and their cases have been followed at our institution since infancy.

The ROP categories are defined as follows: The definitions of the Mild and Moderate categories are similar to those used by others.²¹ "None" indicates that there were no signs of ROP. "Mild" ROP designates ROP in zone II, in stage 2 or less, with no plus disease, or with all ROP first appearing in zone 3. "Moderate" ROP includes zone I or zone II with stage 2+ or stage 3 with less than threshold severity. The retinal vessels of one infant (patient 17) in the Moderate group reached zone III before progressing to 5 clock hours of stage 3 disease in temporal retina; this patient received diode laser treatment to the avascular temporal retina. Also remarkable is another infant (patient 18) in the Moderate group who was small for gestational age. Patients in the "Severe" group had threshold ROP (≥ 5 contiguous clock hours or ≥ 8 cumulative clock hours of stage 3 ROP in the presence of plus disease). "Very Severe" ROP indicates those patients with stage 4 disease that has caused retinal folds and partial or total retinal detachments. All patients in the Severe and Very Severe groups received peripheral diode laser photocoagulation to the avascular retina, with the exception of patients 21 and 23 who were treated with cryotherapy, and patient 25 who had detachments discovered without antecedent threshold disease having been identified. The detachments of patients 22 through 25 had been treated with drainage and buckling. No patient had active ROP or a retinal detachment at the time of the ERG test. The study was approved by the Children's Hospital Committee on Clinical Investigation.

ERG PROCEDURES

The pupil was dilated with 1% cyclopentolate hydrochloride and the child dark adapted for 30 minutes. After dark adaptation, in dim red light, 0.5% proparacaine hydrochloride was instilled and a bipolar Burian-Allen electrode was placed on the left cornea, except in patient 25, who had a long-standing, funnel detachment of the left retina; the healthier right eye was then tested.

Blue (Wratten 47B, $\lambda < 510$ nm; Eastman Kodak Co, Rochester, NY) strobe stimuli (Novatron, Dallas, Tex) were delivered through a 41-cm integrating sphere, controlled in intensity by calibrated neutral-density filters, and ranged

from those evoking a small b-wave ($< 15 \mu\text{V}$) to those that saturated the a-wave amplitude. The unattenuated flash, measured with a detector (S350; United Detector Technology, Orlando, Fla) placed at the position of the subject's cornea, was $3.82 \log \mu\text{W}/\text{cm}^2$ per flash. The scotopic troland value of the stimulus was calculated²²⁻²⁴ by taking each child's pupillary diameter, the average axial length for age,²⁵ and media density^{26,27} into account.

All responses were differentially amplified (bandpass, 1-1000 Hz; gain, 1000), displayed on an oscilloscope, digitized, and stored on disk for analysis later using a Nicolet Compact 4 (Nicolet Biomedical Instruments, Madison, Wis). An adjustable voltage window was used to reject records contaminated by artifacts. Two to 16 responses were averaged in each stimulus condition. The interstimulus interval ranged from 2 to 60 seconds and was selected so that subsequent b-wave amplitudes were not attenuated.

The rod photoreceptor characteristics were calculated from the a-wave responses using the Hood and Birch²⁸ formulation of the Lamb and Pugh model^{12,29} of the biochemical processes involved in the activation of phototransduction. The main parameters of this model are S and R_{mp3} . "S" is a sensitivity parameter, and " R_{mp3} " is the amplitude of the saturated rod response.^{12,29} A curve-fitting routine (MATLAB, fmins) to determine the best fitting values of S, R_{mp3} , and " t_d ," a brief time delay, was used in the equation:

$$(1) \quad R(i,t) = R_{mp3} (1 - \exp[-0.5 S I \{t - t_d\}^2]),$$

where "I" is the flash in isomerizations per rod per flash. Approximately 8.5 isomerizations per rod per flash are produced by 1 scotopic troland second.³⁰ Fitting of the model was restricted to the leading edge of the a-wave response, or to a maximum of 20 milliseconds after stimulus onset. All 3 parameters were free to vary.

For the rod-driven b-wave, which represents mainly the activity of the bipolar cells,^{31,32} the stimulus/response function

$$(2) \quad V/V_{max} = I/(1 + \sigma)$$

was fit to the b-wave amplitudes of each subject using an iterative procedure that minimized the mean square deviation of the data from the equation. In equation 2, "V" is the b-wave amplitude, " V_{max} " is the saturated amplitude, "I" is the stimulus in scotopic troland seconds, and " σ " is the stimulus that evoked a half-maximum b-wave amplitude. Thus, $1/\sigma$ is a measure of sensitivity. The stimulus/response function was fit up to those higher flash intensities at which a-wave intrusion occurs.³³

STATISTICAL ANALYSES

The photoreceptor response parameters S and R_{mp3} of an individual patient with ROP were compared with the normal values for age.³⁴ The patient's value was expressed as a percent of normal for age.³⁴ Analysis of variance was used to test the hypothesis that the photoreceptor response parameters varied significantly with category of ROP. Deficits in photoreceptor sensitivity S were examined for significant correlation with deficits in bipolar cell sensitivity. Deficits in saturated amplitude of the photoreceptor response R_{mp3} were examined for significant correlation with deficits in the saturated b-wave amplitude V_{max} . These parameters will be correlated if deficits in photoreceptor function determine the deficits in bipolar cell response parameters.³⁵

Table 1. Clinical Characteristics*

Patient No.	History			Status of Tested Eye at Time of ERG Test		
	Examination Status	Gestational Age at Birth, wk	Birth Weight, g	Age Postterm	Ophthalmoscopy	Spherical Equivalent, D
ROP						
1	SE	31	1590	9 y	Normal	Plano
2	SE	32	2275	2.5 mo	Normal	+2.00
3	SE	32	1430	18 y	Normal	+2.435
Mild ROP						
4	PR	24	780	2.5 mo	Normal	+4.00
5	PR	25	810	11 y	Normal	-7.50
6	SE	26	730	2.5 mo	Normal	+1.00
7	PR	27	1276	4 mo	Normal	+1.00
8	SE	28	745	2.5 mo	Normal	+3.00
9	SE	28	790	13 y	Normal	-2.00
10	SE	28	1077	14 y	Normal	-0.175
11	SE	28	800	13 y	Normal	-2.00
12	SE	31	1050	2.5 mo	Normal	+2.50
13	PR	31	1300	9 y	Normal	-9.00
Moderate ROP						
14	SE	24	700	4 y	Dragged macula	+1.25
15	PR	24	710	8 mo	Dragged macula	-5.00
16	PR	26	900	7 y	Folds in macula	+6.50
17	SE	27	950	2.5 mo	Laser treatment, temporal retina	+1.00
18	SE	28	450	1 y	Attenuated retinal vessels	+2.50
Severe ROP						
19	PR	25	800	7 mo	Peripheral laser treatment sites	-4.50
20	PR	26	499	6 mo	Peripheral laser treatment sites	-0.50
21	PR	28	1106	8 y	Peripheral cryotherapy sites	-1.25
Very severe ROP						
22	SE	24	730	1 y	Peripheral laser treatment sites	-16.00
23	SE	24	694	6 y	Peripheral cryotherapy sites	+0.25
24	SE	24.5	770	4 y	Peripheral laser treatment sites	-5.00
25	SE	26.5	830	1 y	Peripheral laser treatment sites	-3.50

*ERG indicates electroretinographic; D, diopters; ROP, retinopathy of prematurity; SE, standard examination; and PR, previously reported.

RESULTS

Sample records (**Figure 1**) and a-wave and b-wave model fits (**Figure 2**) for patient 6 (Table 1) and a normal, term born control infant show that a-wave and b-wave responses of the ROP patient are attenuated. In Figure 2, the upper panels show the first 40 milliseconds of the responses on an expanded time scale, and the fit of equation 1 (dashed lines) to the leading edge of the a-wave. In the lower panels of Figure 2, b-wave amplitude is plotted as a function of stimulus intensity; the fit of equation 2 to the data is shown by the dashed curve. The b-wave responses to higher flash intensities, at which a-wave intrusion occurs,³³ are not included in the fits. The patients' values of S, R_{mp3} , $\log \sigma$, and V_{max} are presented in **Table 2** along with the normal values. Patients 22 through 25, whose response amplitudes were not sufficient for these analyses, are not included in Table 2.

In **Figure 3**, S is expressed as a percentage of normal for age³⁴ and grouped according to ROP category. For the None, Mild, Moderate, and Severe groups, S varies significantly with ROP category for analysis of variance ($F_{3,17}=9.28, P<.01$). The deficits in R_{mp3} also vary significantly with ROP category using the analysis of variance

($F_{3,17}=9.68, P<.01$). The brief delay, t_d , in patients was within the range found in healthy subjects.

For the 3 in the None category, both S and R_{mp3} are within the 95% prediction limit³⁶ for normal.³⁴ In the Mild group, 4 infants (Table 1, patients 4, 9-11), including 1 tested at age 2.5 months' postterm, were normal for age,³⁴ and 6 (Table 1, patients 5-8, 12, 13) are only approximately 50% (range, 32%-55%) of normal for age. Of these, the 2 (Table 1, patients 5 and 13) who were tested after infancy had become high myopes before age 2 years⁴; the 4 who were tested at 2.5 months postterm were not myopic (Table 1) although only 1 (patient 4) had values for S and R_{mp3} that were normal for age (Table 2). In the Moderate and Severe groups, both S and R_{mp3} were approximately 50% (range 36%-66%) of the normal for age. For patients in the Very Severe category, whose markedly attenuated ERG responses precluded fits of the a-wave and b-wave models, responses to a blue flash producing retinal illumination of approximately 10^3 log scotopic troland seconds were detectable, but less than a third (11-207 μV) of the normal mean amplitude (627 μV ; SD=144 μV ; n=25).

If departures of b-wave parameters from normal are accounted for completely by abnormal photoreceptor inputs to the rod-driven bipolar cells,³⁵ the points in

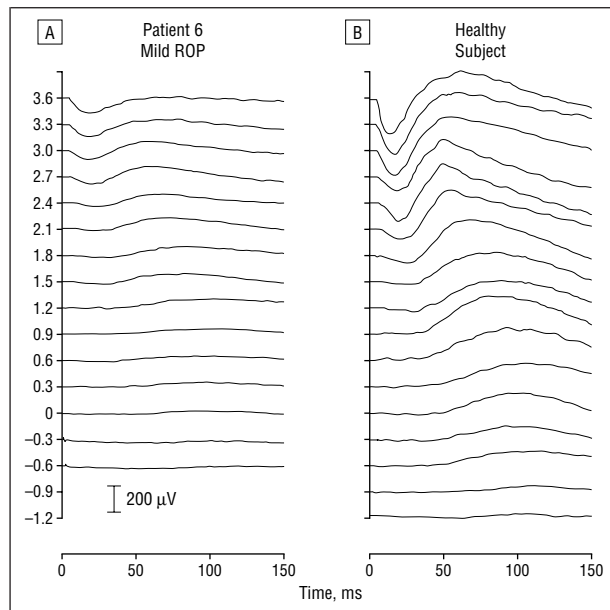


Figure 1. A, Sample electroretinographic record at 2.5 months' postterm in patient 6 with Mild retinopathy of prematurity (ROP). B, Electroretinographic record of a healthy, term born infant. The numbers to the left of each trace indicate the stimulus in log scotopic troland seconds.

Figure 4, which show the relation of a-wave to b-wave parameters, would lie on the diagonal lines. The deficits in the b-wave sensitivity parameter, σ , are correlated ($r=0.44$; $P<.05$) with deficits in S (Figure 4, A). In Figure 4B, the correlation ($r=0.84$; $P<.01$) of the saturated b-wave amplitude, V_{max} , and R_{mp3} is shown. Thus, in these patients, departures of the b-wave response parameters from normal can be accounted for by rod photoreceptor dysfunction.³⁵ None of the a-wave or b-wave parameters vary significantly with gestational age at birth.

COMMENT

These data demonstrate a significant association of rod photoreceptor dysfunction and ROP. The compromise in photoreceptor function varies significantly with the severity of acute phase ROP (Figure 3). The deficits in the responses of the photoreceptors are sufficient to account for the b-wave response parameters (Figure 4). The rod cell dysfunction represented by deficits in S and R_{mp3} are neither explained by prematurity alone nor by photocoagulation alone. The response parameters S and R_{mp3} (Table 2) are normal for age³⁴ in the former preterm infants who had no ROP. Photocoagulation alone does not explain the results. Six infants (patients 5-8, 12, 13) in the Mild group and 4 infants (patients 14-16, 18) in the Moderate group who received no ROP treatment whatsoever have response parameters (Table 2) below normal for age.³⁴

The rod response parameters summarize the molecular processes involved in the activation of rod phototransduction.^{12,29} The sensitivity parameter, S , reflects the cascade of events from photon capture up to, and including, closure of the cyclic guanosine monophosphate-regulated channels in the outer segment plasma membrane. The amplitude of the satu-

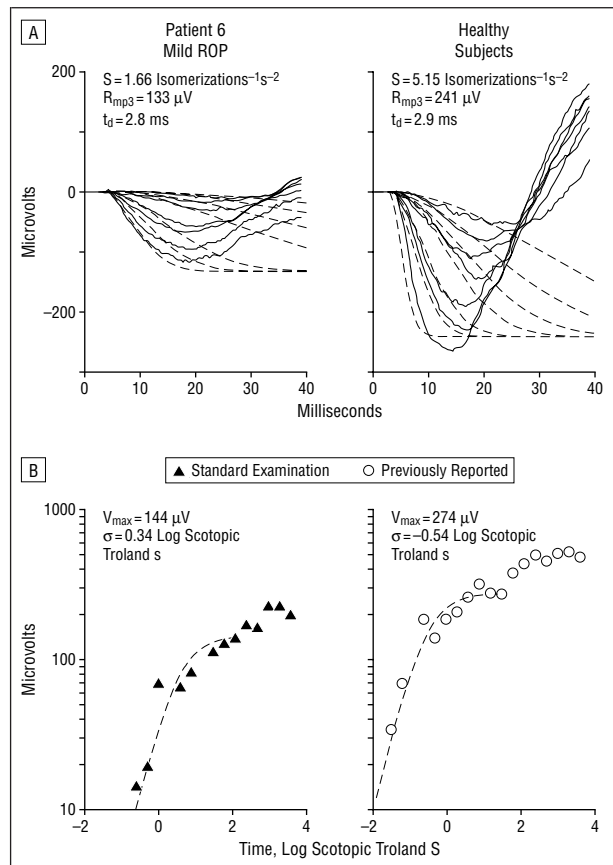


Figure 2. For records of patient 6, the dashed lines show the fits of the equation $R(i,t)=R_{mp3}(1-\exp[-0.5 S I (t-t_d)^2])$ to the a-wave (A) and of the equation $V/V_{max}=I/(I+\sigma)$ to the b-wave data (B). ROP indicates retinopathy of prematurity; R_{mp3} , the amplitude of the saturated rod response; V , b-wave amplitude; V_{max} , the saturated b-wave amplitude; S , sensitivity parameter for the rod photoreceptor response; I , stimulus in scotopic troland seconds; t_d , a brief time delay; and σ , the half-maximum b-wave amplitude evoked stimulus.

rated response from the photoreceptors, R_{mp3} , reflects the number of channels in the outer segment membrane that are available for closure by light. Short outer segments, a low amount of rhodopsin and consequent diminished quantum catch, impaired mobilities of the transduction cascade proteins (rhodopsin, transducin, and phosphodiesterase) in the disc membranes, and abnormal disc-to-channel relations are nonmutually exclusive explanations for low values of S and R_{mp3} . In a rat model of ROP, rhodopsin content was not low, but disorganization of the outer segments explained the low values of S and R_{mp3} . The outer segment abnormalities rendered the stimuli less effective at evoking photoreceptor responses.^{6,17}

The significant association of rod photoreceptor dysfunction and severity of ROP do not distinguish cause and effect. However, we note that in animal models of ROP, structural abnormalities of the outer segments,⁶ photoreceptor dysfunction,¹⁷ and expression of a gene that causes photoreceptor disease³⁷ all antedate the appearance of the retinal vascular changes that define clinical ROP.^{1,19} Thus, the photoreceptors' high demands for oxygen and energy may contribute to the retinal hypoxia that leads to ROP.

Table 2. Electroretinographic Parameters*

No.	Examination Status	Rod Photoreceptors (a-Wave) Equation 1			Rod-Driven Bipolars (b-Wave) Equation 2	
		S (Isomerizations ⁻¹ s ⁻²)	R _{mp3} (μV)	t _d (ms)	Log σ (Scotopic Troland s)	Vmax (μV)
No ROP						
1	SE	12.10	233	4.0	-1.0	433
2	SE	9.06	332.2	3.4	-0.12	203
3	SE	11.60	310	4.1	-0.86	372
Mild ROP						
4	PR	4.94	85	4.4	-0.41	225
5	PR	4.79	287	2.3	-0.63	315
6	SE	1.66	133	2.8	0.34	144
7	PR	3.04	126	3.3	-0.17	340
8	SE	2.56	213	3.2	-0.52	263
9	SE	10.56	370	3.6	-1.04	361
10	SE	9.98	310	3.7	-0.36	408
11	SE	9.33	379	3.7	-0.85	400
12	SE	2.07	143	2.4	0.33	152
13	PR	4.26	136	6.3	-0.24	320
Moderate						
14	SE	4.58	76	3.0	-0.35	134
15	PR	4.45	150	3.3	-0.02	142
16	PR	4.78	88	3.0	-0.08	126
17	SE	1.77	243	2.8	-0.25	190
18	SE	5.92	33	2.0	0.40	47
Severe						
19	PR	3.16	155	3.4	-0.60	257
20	PR	2.60	106	3.3	-0.47	167
21	PR	5.54	76	3.4	-0.75	71
Age 2.5 mo (n = 27)		5.02 (0.19) [1.63 to 8.41]	161 (51) [54 to 268]	3.6 (0.7) [2.1 to 5.1]	-0.38 (0.18) [0 to -0.76]	194 (62) [64 to 324]
Children and adults (n = 24)		10.2 (0.19) [6.79 to 13.61]	383 (75) [225 to 541]	3.4 (0.5) [2.3 to 4.5]	-0.84 (0.10) [-0.63 to -1.05]	379 (59) [254 to 504]

*Equation 1 indicates $R(i, t) = R_{mp3}(1 - \exp[-0.5 S I (t - t_d)^2])$; equation 2, $V/V_{max} = I/(I + \sigma)$; ROP, retinopathy of prematurity; SE, standard examination; PR, previously reported. For explanation of the abbreviations in these equations, see the legend to Figure 2.

†All data for "Term-born controls" are presented as means (SDs) [95% confidence intervals].³⁴

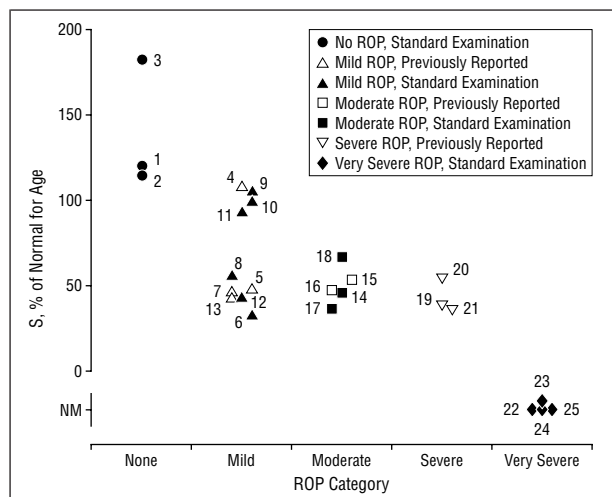


Figure 3. Rod photoreceptor sensitivity, S, is grouped according to retinopathy of prematurity (ROP) category. Each plotted point represents an individual patient; patient numbers are also shown. S varies significantly with ROP category.

Although outer segment abnormalities can account for the ERG results, the primary insult to the rods is unlikely to strike the outer segments directly. Mammalian outer segments are turned over and completely

renewed approximately every 10 days.¹⁶ Therefore, in the children, and even in the infants (Table 1), outer segments have turned over many times between the age at which ROP was active and the age at which the ERG was recorded. To produce the long-term effects on the outer segments indicated by these ERG results, we suspect that the events that lead to ROP alter synthesis of the outer segment discs and the cytoskeleton of the rod photoreceptors. Indeed, in a mouse model of ROP, Pierce et al³⁷ have found that the gene for dominant retinitis pigmentosa, *Rp1*, which is rapidly regulated by retinal oxygen status, is expressed in the photoreceptor inner segments and cell bodies. Additionally, it is noted that *Rp1* is upstream of several photoreceptor-specific genes, including those for opsin and arrestin, and the *Rp1* protein has a region of homology with the *Drosophila* protein BIF that is required for normal photoreceptor morphogenesis.³⁷ It remains to be determined what, if any, role *Rp1* or other genes expressed in the photoreceptors have in human ROP.

No matter what the molecular cause of the alterations in the rod photoreceptors of the patients with ROP, the low rod sensitivity in some of the patients (Figure 2) has implications for rod-mediated vision. In ROP subjects, elevation of dark-adapted thresholds and altered adaptation to steady background lights are

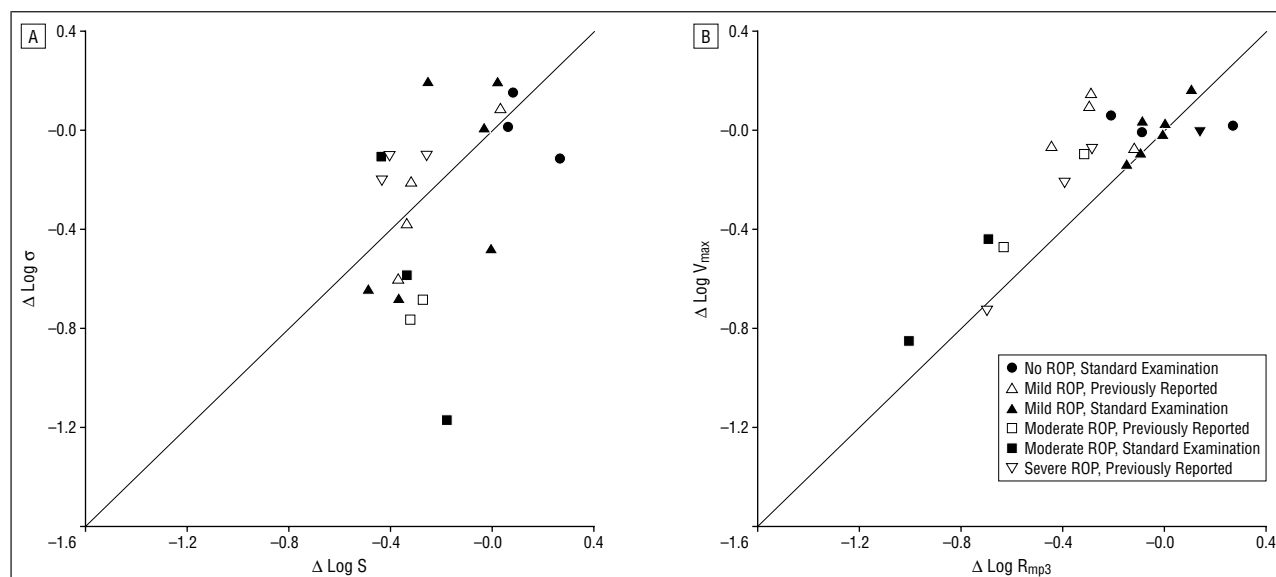


Figure 4. A, Deficits in the rod sensitivity parameter, S , are correlated with deficits in the b-wave sensitivity parameter, σ . B, Deficits in the amplitude of the saturated rod response, R_{mp3} , are correlated with the saturated amplitude of the b-wave response, V_{max} .

attributable to rod dysfunction.^{4,5} There are regional variations in rod-mediated visual sensitivity, which have significant associations with early high myopia in mild ROP.^{4,5} Because the retina, including the photoreceptors, is involved in the control of eye growth,³⁸⁻⁴¹ the alterations in the retinal function that can be analyzed in ERG studies may be involved in the deregulation of eye growth and development of refractive errors that are so common in these (Table 1) and other patients with ROP.^{3,21,42}

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Answer to Crossword Puzzle

The Crossed Eye Richard D. Key, MD

This is the answer to the March crossword puzzle (*Arch Ophthalmol*. 2001;119:463). Answers are immediately available at www.archophthalmol.com.

1	I	2	S		3	A	4	N	5	I	6	R	7	I	8	D	9	I	10	A													
11	O	11	H		12	L	12	E	13	N	13	T	14	I	14	C	15	U	15	L	16	A	17	R									
15	P	15	U	16	N	16	C	17	T	17	A					18	D	18	E	19	U	19	X										
		18	T	18	A	19	O	19	S	20		20	D	21	A	21	B	22	U	22	G												
23	S	23	E	24	A	24	N	25		25	R	26	O	26	S	27	E	27	T	28	T	28	S										
27	A	27	Y	28	N	28	T	29		29	K	30	A	30	G	31	E	31	S	32		32	A										
31	H	31	E	32		32	R	33		33	O	34		34		35	I	35	E	36		36	H	37	L								
35	I	35		36		36	L	37		37	O	38		38	B	39	U	39	N	40		40	G	41	O	42	A						
40	B	40	E	41		41	A	42		42	M	43		43	M	44	E	44	U	45		45	P	46	K	47	U	48	R	49	D		
		44	T	44	S	45		45		46	Y	46		46	E	47	S	47		47		48	B	48	O	49	N	50	N				
47	C	47	O	48	S	48	I	49		49		50		50	M	51	A	51		51		52	R	52	N	53	E	54	R				
51	A	51	N	52	I	52	S	53		53	O	54		54	C	55	O	55		55		56	R	56	I	57	A	58	T	59	O		
		56		56		57	E	57		58	M	58		58	I	59	S	59		59		60	S	60	I	61	O	62	N	63	S	64	D