Increment Threshold Functions in Retinopathy of Prematurity

Ronald M. Hansen, Anne Moskowitz, Jennifer N. Bush, and Anne B. Fulton

Department of Ophthalmology, Boston Children's Hospital & Harvard Medical School, Boston, Massachusetts, United States

Correspondence: Ronald M. Hansen, Department of Ophthalmology, Boston Children's Hospital & Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA; ronald.hansen@childrens.harvard.edu.

Submitted: February 4, 2016
Accepted: March 29, 2016

Citation: Hansen RM, Moskowitz A, Bush JN, Fulton AB. Increment threshold functions in retinopathy of prematurity. *Invest Ophthalmol Vis Sci*. 2016;57:2421–2427. DOI:10.1167/iovs.16-19294

Purpose. To assess scotopic background adaptation in subjects with a history of preterm birth and retinopathy of prematurity (ROP). Retinopathy of prematurity is known to have long-term effects on rod photoreceptor and rod mediated postreceptor retinal function.

Methods. Rod-mediated thresholds for detection of 3° diameter, 50 ms stimuli presented 20° from fixation were measured using a spatial forced choice method in 36 subjects (aged 9–17 years) with a history of preterm birth and 11 age similar term-born subjects. Thresholds were measured first in the dark-adapted condition and then in the presence of 6 steady background lights (−2.8 to +2.0 log scot td). A model of the increment threshold function was fit to each subject's thresholds to estimate the dark-adapted threshold (T_{DA}) and the *Eigengrau* (A_0, the background that elevates threshold 0.3 log unit above T_{DA}).

Results. In subjects with a history of severe ROP, both T_{DA} and A_0 were significantly elevated relative to those in former preterms who never had ROP and term-born control subjects. Subjects who had mild ROP had normal T_{DA} but elevated A_0. Neither T_{DA} nor A_0 differed significantly between former preterms who never had ROP and term-born controls.

Conclusions. The results suggest that in severe ROP, threshold is affected at a preadaptation site, possibly the rod outer segment. In mild ROP, changes in the *Eigengrau* may reflect increased intrinsic noise in the photoreceptor or postreceptor circuitry or both.

Keywords: retinopathy of prematurity, increment threshold, retinal adaptation, photoreceptors, visual psychophysics

The onset of retinopathy of prematurity (ROP) occurs at preterm ages during a period of rapid development of the rod photoreceptor outer segments.[1] Electroretinographic studies of infants with a history of ROP found significant deficits in both rod photoreceptor (a-wave) and rod-mediated postreceptor (b-wave) sensitivity compared with term-born control infants.[1] In electroretinographic studies of children and adolescents, those with a history of mild ROP showed persistent deficits in rod photoreceptor sensitivity but normal postreceptor sensitivity, which we interpreted as evidence of remodeling of the postreceptor retina.[2] In those with severe ROP, both photoreceptor and postreceptor sensitivity remained abnormal.[2]

Convergence of signals from rods to postreceptor cells is fundamental to retinal organization and plays a role in determining visual threshold.[3,4] We found that the critical area for complete spatial summation in ROP subjects was significantly larger than in controls.[5] Thus, increased spatial pooling of rod input to postreceptor circuitry may be a factor involved in the normalization of sensitivity in mild ROP subjects.[5]

To probe further into rod-mediated function in ROP, we measured visual thresholds in subjects with a history of preterm birth first in the dark and then in the presence of steady background lights. Dim backgrounds have little effect on threshold; as background intensity is increased, thresholds are elevated according to Weber's law.[6–8] The resulting increment threshold functions can be analyzed to assess the effects of ROP on receptor and postreceptor function.[9,10] Adaptation to changing levels of illumination requires adjustment of retinal signals in photoreceptors and postreceptor neural cells.[4,6,11]

Methods

Subjects

Thresholds were measured in 36 subjects with a history of preterm birth (Table). Gestational age at birth ranged from 24 to 32 weeks (median: 27 weeks) and birthweight from 530 to 2070 g (median: 998 g). Although, on average, those with severe ROP were born earlier and had lower birthweight, there was considerable overlap among the preterm groups. All subjects had serial fundus examinations in the newborn intensive care nursery following a schedule similar to that used in multicenter clinical trials.[12] In the international classification of retinopathy of prematurity (ICROP) classification of active ROP, location of the retinopathy is specified by zone, severity by stage, and extent by clock hours.[13] Retinopathy of prematurity is an active disease at preterm ages and resolves by the early postterm weeks.[14] We categorized each subject based on the results of examinations in the nursery according to maximum severity of acute-phase ROP as severe ROP ($n = 10$); mild ROP ($n = 14$); or no ROP ($n = 12$). Those in the severe category had been treated by laser ablation of avascular peripheral retina. In all severe ROP subjects, the retina was clinically healed; the region of treated retina was peripheral to the 20° site of the test stimuli. The maximum severity was stage 3; three had zone I disease and none had

Investigative Ophthalmology & Visual Science

TABLE. Subject Characteristics, Median (Range)

Group	*n*	Gestational Age, wk	Birthweight, g	Age at Test, y	logMAR VA OU	Spherical Equivalent, Diopters	
						OD	OS
No ROP	12	30.0 (26.0 to 32.0)	1587 (715 to 2070)	13.5 (11.8 to 17.8)	0.00 (0.12 to −0.24)	+0.22 (−4.63 to +0.75)	+0.25 (−6.38 to +1.13)
Mild ROP	14	26.5 (24.0 to 29.0)	893 (535 to 1375)	13.9 (10.5 to 17.4)	−0.05 (0.30 to −0.18)	+0.03 (−3.38 to +2.06)	+0.31 (−3.13 to +1.75)
Severe ROP	10	24.5 (24.0 to 28.0)	648 (530 to 1000)	13.3 (9.8 to 16.5)	0.19 (0.80 to −0.06)	−5.31 (−19.13 to −2.00)	−4.13 (−19.00 to −0.75)

VA, visual acuity.

retinal detachment. Those in the mild category had ROP that, by clinical criteria, resolved completely without treatment. Their maximum severity of ROP was stage 1 or 2 in zone II or III.[13] In all 24 participants who had ROP, the disease had been symmetric in the two eyes. Those in the no ROP category had serial examinations and ROP was never detected. The subjects ranged in age from 9 to 17 years (median: 13.5 years) at the time of testing. A total of 11 healthy, term-born control subjects aged 10 to 17 years (median: 12.8 years) also participated.

The study conformed to the tenets of the Declaration of Helsinki and was approved by the Children's Hospital Committee on Clinical Investigation. Written informed consent was obtained from the parents and assent from the children before each session.

Procedure

Rod-mediated thresholds for detection of 50 ms, 3° diameter, blue (Wratten 47B, $\lambda < 440$ nm) spots were obtained using a two-alternative, spatial-forced choice procedure. A red light-emitting diode (LED) fixation target that subtended 30 min arc and flickered at 1 Hz was at the center of the screen. Stimuli were presented on a rear projection screen 20° to the right or left of the fixation target. For the background conditions, a steady red (Wratten 29, $\lambda > 610$ nm) circular, 90° diameter field, concentric with the fixation target, was shown on the screen. Calibrated neutral density filters controlled the intensity of the test and background lights.

After 30 minutes of dark adaptation, the subject sat 50 cm in front of the rear projection screen and viewed the screen with both eyes. On each trial, the subject was asked to look at the fixation target and to report the position (right or left) of the test stimulus. The subject received feedback on every trial. Threshold was measured using a transformed up-down staircase that estimated the 70.7% correct point of the psychometric function.[15] At least five alternations (consisting of two reversals) of response type (correct, incorrect) were obtained. Subjects were tested first in the dark-adapted (no background) condition and then in the presence of each of a series of six backgrounds that produced retinal illuminance of approximately −2.8 to +2.0 log scot td.

Calibrations

The luminance of the test and background lights was measured with a calibrated photodiode (IL 1700; International Light, Newburyport, MA, USA) placed in the position of the subject's eyes. The scotopic troland values of the stimuli were calculated taking each subject's measured pupil diameter into account.

Analyses

A model[9,10] of the increment threshold function (Fig. 1) was fit to each subject's thresholds to calculate the values of dark-adapted threshold (T_{DA}) and *Eigengrau* (A_0) that minimized the sum of squared deviations from:

$$\log T = \log T_{DA} + \log[1 + (I/A_0)] \qquad (1)$$

where T is the threshold at background intensity I, A_0 is the background that raises the threshold 0.3 log unit above the dark-adapted threshold and approaches estimated values of intrinsic retinal noise.[4,11,16–18]

According to classical psychophysical theory,[9,10,19] a disease that affects the rod photoreceptor distal to the site of adaptation (presumably the rod outer segment) and reduces the ability of the rod to absorb photons or to produce an input to the postreceptor retina will affect both the test and background light equally. Equal reduction of quantum catch

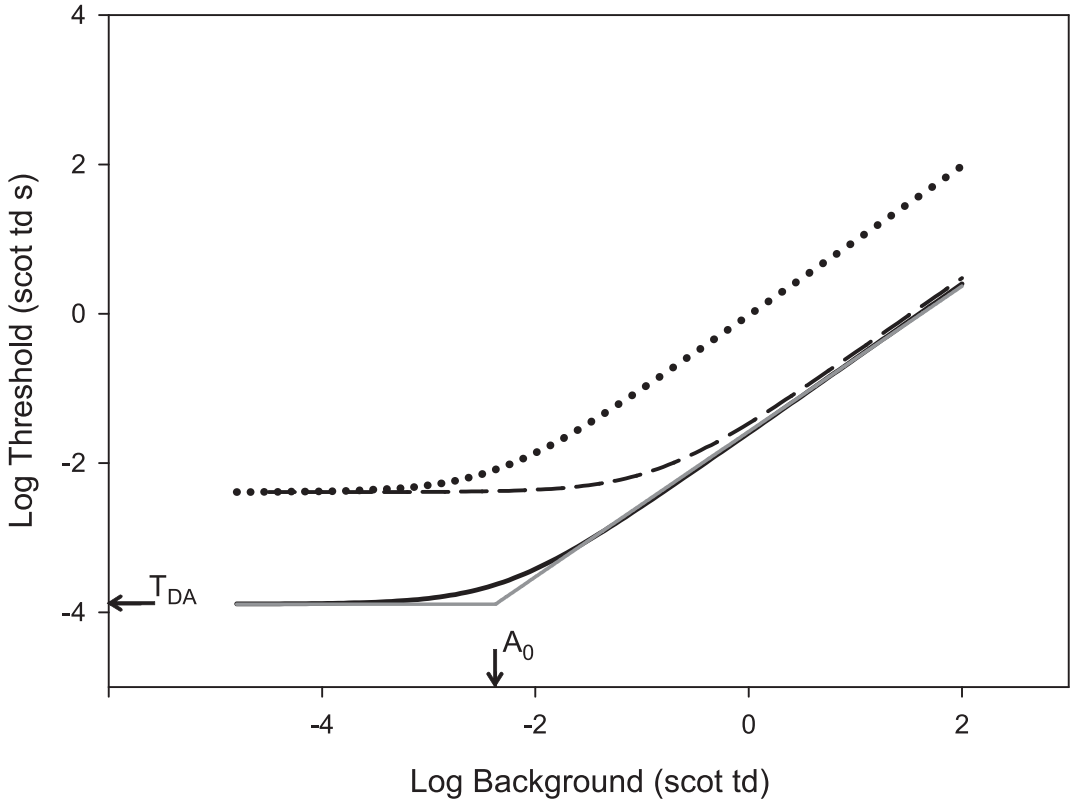

Figure 1. Model of the rod increment threshold function. The *solid curve* is a plot of Equation 1. The dark-adapted threshold (T_{DA}) and *Eigengrau* (A_0) values of a typical control subject are indicated by the arrows. The horizontal asymptote is at T_{DA}; the *oblique asymptote* (slope = 1.0) intersects T_{DA} at A_0, the background that elevates threshold by a factor of 2 (0.3 log units). Disease affecting the retina distal to the site of adaptation causes the curve (*dasbes*) to shift up and to the right; T_{DA} and A_0 are shifted by equal amounts. Disease affecting a site at or after the site of adaptation causes the curve (*dots*) to shift up with no horizontal shift; T_{DA} is shifted but A_0 is not.

from both the test and background stimuli produces equal elevation of both T_{DA} and A_0. Plotted on log-log coordinates, the increment threshold function is shifted both vertically and horizontally by equal amounts (Fig. 1, dashed curve). A disease that affects the more proximal retinal pathways, at or after the site of adaptation, reduces effectiveness of the test flash but not of the background; the increment threshold function is shifted vertically but not horizontally (Fig. 1, dotted curve).

Analysis of variance was used to evaluate T_{DA} and A_0 for significant differences among the groups (severe ROP, mild ROP, no ROP, and term-born). The Scheffé test was used in post-hoc comparisons. The parameter T_{DA} of the model was compared with the actual dark-adapted threshold using repeated measures analysis of variance. For all tests, the level of significance was $P \leq 0.01$.

Results

Increment threshold functions from a representative subject of each group are shown in Figure 2. These subjects had T_{DA} near the median value for their group. To assess goodness of fit of Equation 1, we calculated RMS errors for each subject and compared across groups using analysis of variance. There was no significant difference in goodness of fit between groups ($F = 2.15$; *df*: 3, 43; $P = 0.108$). We also calculated standard errors for the parameters T_{DA} and A_0 for each subject. Analysis of variance showed that the standard errors did not vary significantly among the groups (T_{DA}: $F = 2.59$; *df*: 3, 43; $P = 0.065$; A_0: $F = 0.675$; *df*: 3, 43; $P = 0.572$).

The values of the calculated parameters T_{DA} and A_0 are shown for every subject in Figure 3. Analysis of variance showed that T_{DA} varied significantly between groups ($F = 13.9$; *df*: 3, 43; $P < 0.001$). Results of Scheffé tests indicated that T_{DA} values in mild ROP, no ROP, and term-born subjects did not differ significantly but T_{DA} in severe ROP subjects was significantly higher than in the other groups. The *Eigengrau* (A_0) also varied significantly with group ($F = 31.8$; *df*: 3, 43; $P < 0.001$). Post hoc tests showed that A_0 values in severe ROP subjects were significantly higher than in mild ROP subjects; both of these values were significantly higher than A_0 values in the no ROP and term-born groups. Values of A_0 in the no ROP and term-born subjects did not differ from each other and were within the range reported in other psychophysical studies of healthy mature subjects.[4,16,20] The measured dark-adapted threshold did not differ significantly from the calculated value, T_{DA}, in any group ($F = 0.95$; *df*: 1, 43; *P*: ns).

Figure 4 plots deficits in T_{DA} as a function of deficits in A_0 for each subject. The deficit for each subject is expressed relative to the mean for each of the parameters in term-born control subjects. Of the severe ROP subjects, 9 of the 10 show approximately equal changes in T_{DA} and A_0; their points lie close to the diagonal. This is consistent with ROP affecting a preadaptation site. One severe ROP subject had a T_{DA} value in the normal range but elevated A_0; his ICROP classification and treatment were similar to that of others in his group. Most of the mild ROP subjects (12 of 14) had A_0 values that exceed the normal range, consistent with increased retinal noise either in the photoreceptor, the postreceptor neural circuitry, or both. Although T_{DA} in the mild group did not differ significantly from

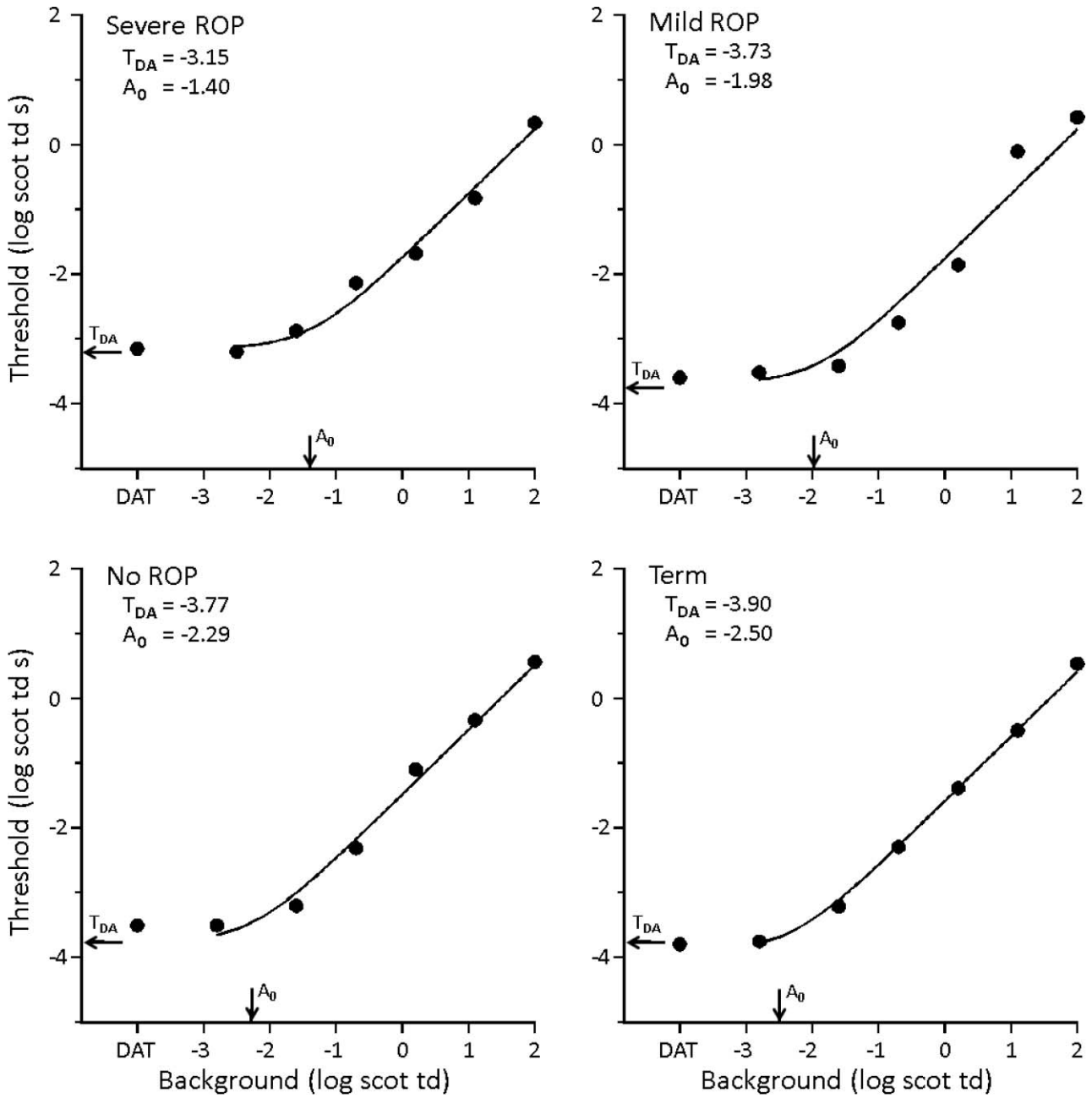

Figure 2. Representative increment threshold functions. Log threshold is plotted as a function of log background for subjects from the three groups of former preterm subjects: severe ROP, mild ROP, and no ROP. Data from a term-born control subject are also shown. In each panel, the model parameters T_{DA} (in log scot td s) and A_0 (in log scot td) are indicated.

the no ROP and term-born groups (Fig. 3, upper panel), six mild ROP subjects had deficits in T_{DA} that exceeded the normal range, perhaps a consequence of low rod photoreceptor sensitivity.[1,2] The severity of ROP in these six subjects was not worse than in others in the mild ROP group.

Discussion

The present results add to the previously reported evidence[1,2,5,21] that ROP has long-lasting effects on the neurosensory retina and provide new evidence of postreceptor retinal dysfunction in mild ROP. Retinopathy of prematurity, whether mild or severe, perturbs photoreceptor and postreceptor function long after active ROP has resolved. Preterm birth alone has little effect on rod system adaptation; increment threshold functions of subjects in the no ROP group are normal and indistinguishable from those in term-born controls.

In severe ROP, the dark-adapted threshold (T_{DA}) and *Eigengrau* (A_0) are elevated by equal amounts. The changes in quantum catch delivered by the test and by the background are equal. This produces a diagonal shift in the function as displayed on log-log coordinates. The diagonal shift that we found in severe ROP subjects (Fig. 4) indicates alteration at a preadaptation site, perhaps the rod outer segment. The range of backgrounds used in the present study is expected to have little effect on sensitivity of the rod photoreceptor.[17,22]

Investigative Ophthalmology & Visual Science

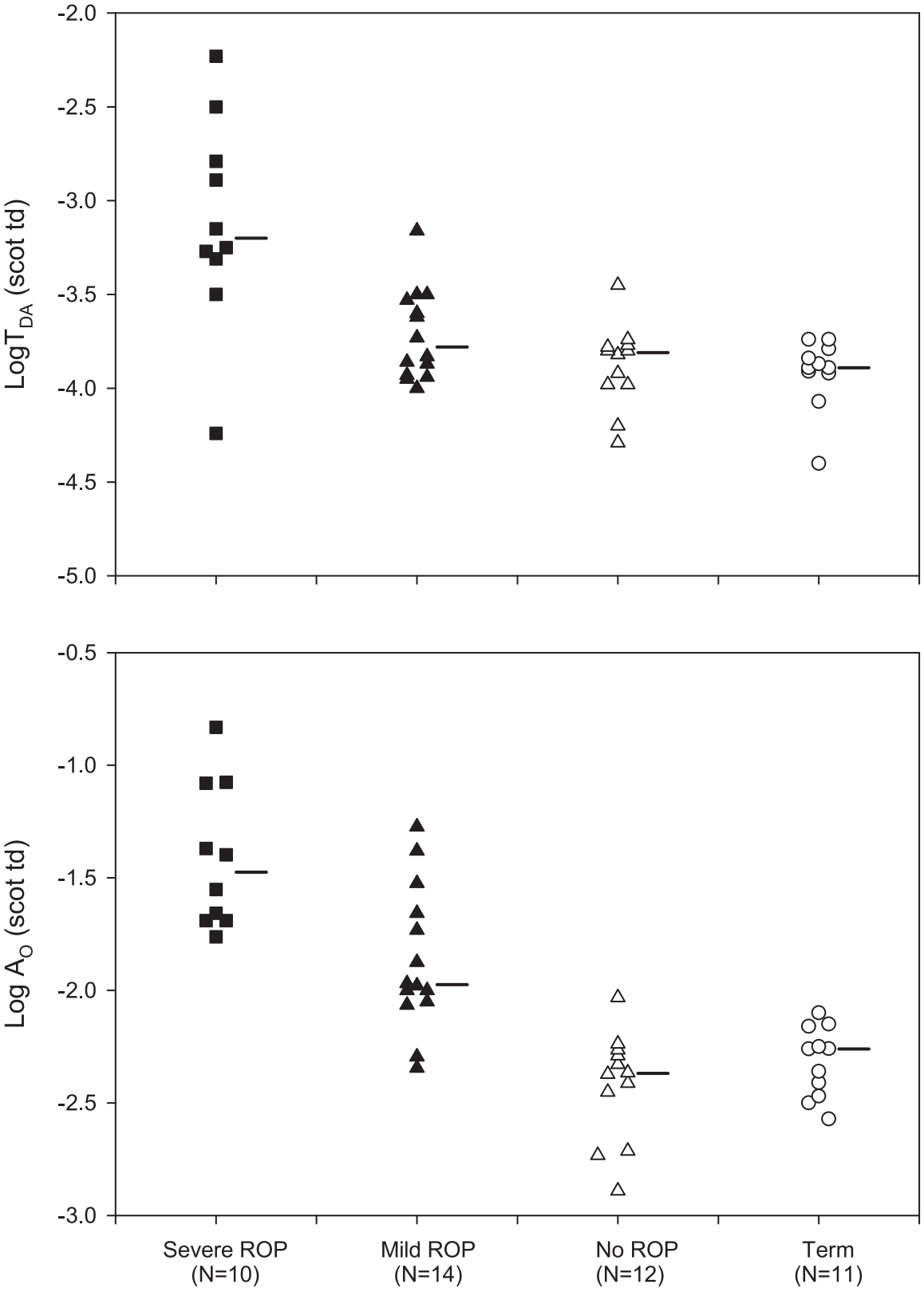

Figure 3. Dark-adapted threshold (T_{DA}) and *Eigengrau* (A_0). *Upper panel*: T_{DA} values in severe ROP, mild ROP, no ROP, and term-born control groups. *Lower panel*: A_0 values in the groups. In both panels, each subject is represented by a point and the *horizontal bars* indicate the median value for each group. The number of subjects in each group is indicated.

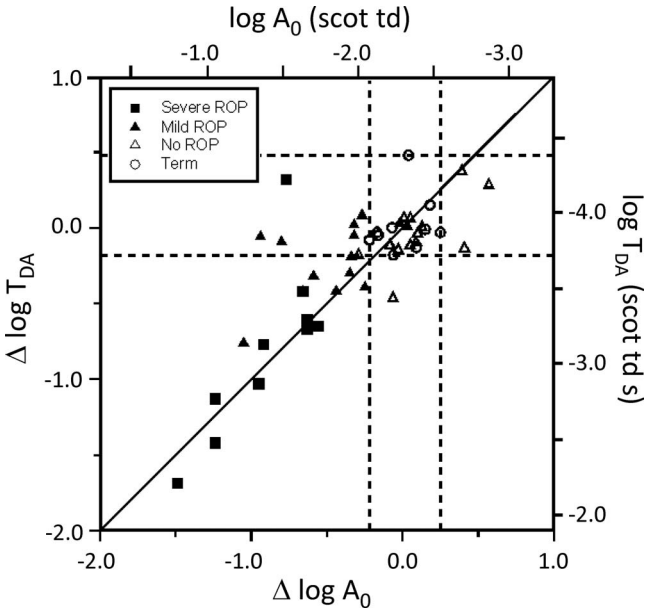

Figure 4. Deficit in T_{DA} values were plotted as a function of deficit in the A_0 for each subject. Each subject is represented by a point. The median value for term-born controls is at (0, 0); the *horizontal dashed lines* indicate the range of T_{DA} and the *vertical dashed lines* indicate the range of A_0 in the term-born controls. The *solid diagonal line* (slope = 1.0) is the prediction for a preadaptation site effect. The values of A_0 and T_{DA} in absolute units are indicated on the *upper* and *right axes*, respectively.

Reduced quantum catch secondary to disorganized rod outer segments, shortened outer segments, or inefficient phototransduction may account, in part, for our results. These explanations are not necessarily mutually exclusive. We previously reported[1,2] that rod photoreceptor sensitivity and saturated response amplitude, estimated from the a-wave of the electroretinogram,[23] remain low in children and adolescents with a history of ROP. Specifically, median rod sensitivity was less than 50% of normal and saturated response amplitude was approximately 40% of normal in children aged 9 to 18 years with a history of severe ROP.[1,2] Furthermore, we have previously reported disorganized and dysmorphic rods, despite an abundance of rhodopsin, in a rat model of ROP.[24]

In mild ROP, the T_{DA} value was normal in most subjects, perhaps a consequence of increased spatial pooling[5] of the remodeled postreceptor retinal circuitry. The *Eigengrau* (A_0) was abnormal even if T_{DA} was normal (Fig. 3). The value of A_0 depends on noise arising in the photoreceptor and the postreceptor retinal circuitry,[25] as well as on temporal and spatial summation of retinal signals.[26] Both spatial and temporal summation are abnormal in mild ROP.[5,21] Perhaps noise in the postreceptor retinal circuitry is elevated in the remodeled retina[2] of mild ROP subjects.

The present results show that ROP has significant long-term effects on function of rod photoreceptors and postreceptor neural circuitry.[1,2,5,21] Our increment threshold results confirm that the deficit in dark-adapted sensitivity, inferred from ERG a-wave results, is located before the site of adaptation, probably at the rod outer segment. In mild ROP, although the dark-adapted threshold is normal, there is evidence of higher than normal intrinsic retinal noise. Thus, the results herein add to the mounting evidence of the importance of the neurosensory retina in the ROP disease process.

Acknowledgments

We thank Theodore Bowe, Garima Soni, and Ashlyn Wiebalck for their assistance.

Supported by National Eye Institute Grant R01 EY010597 and Massachusetts Lions Eye Research Fund.

Disclosure: **R.M. Hansen**, None; **A. Moskowitz**, None; **J.N. Bush**, None; **A.B. Fulton**, None

References

1. Fulton AB, Hansen RM, Moskowitz A, Akula JD. The neurovascular retina in retinopathy of prematurity. *Prog Retin Eye Res*. 2009;28:452–482.
2. Harris ME, Moskowitz A, Fulton AB, Hansen RM. Long-term effects of retinopathy of prematurity (ROP) on rod and rod-driven function. *Doc Ophthalmol*. 2011;122:19–27.
3. Barlow HB, Mollon J. Psychophysical measurement of visual performance. In: Barlow HB, Mollon J, eds. *The Senses*. Cambridge: Cambridge University Press; 1982:114–132.
4. Frishman LJ, Reddy MG, Robson JG. Effects of background light on the human dark-adapted electroretinogram and psychophysical threshold. *J Opt Soc Am A Opt Image Sci Vis*. 1996;13:601–612.
5. Hansen RM, Tavormina JL, Moskowitz A, Fulton AB. Effect of retinopathy of prematurity on scotopic spatial summation. *Invest Ophthalmol Vis Sci*. 2014;55:3311–3313.
6. Shapley R, Enroth-Cugell C. Visual adaptation and retinal gain controls. *Prog Retin Res*. 1984;3:263–346.
7. Hood DC, Finkelstein MA. Sensitivity to light. In: Boff KR, Kaufman L, Thomas J, eds. *Handbook of Perception, Sensory Processes and Perception*. New York, NY: Wiley; 1986:14–22.
8. Sharpe LT. The light adaptation of the human rod visual system. In: Hess RF, Sharpe LT, Nordby K, eds. *Night Vision; Basic, Clinical and Applied Aspects*. Cambridge: Cambridge University Press; 1990:49–124.
9. Hood DC. Testing hypotheses about development with electroretinographic and incremental-threshold data. *J Opt Soc Am A*. 1988;5:2159–2165.
10. Hood DC, Greenstein V. Models of the normal and abnormal rod system. *Vision Res*. 1990;30:51–68.
11. Dunn FA, Doan T, Sampath AP, Rieke F. Controlling the gain of rod-mediated signals in the Mammalian retina. *J Neurosci*. 2006;26:3959–3970.
12. Early Treatment For Retinopathy Of Prematurity Cooperative Group. Revised indications for the treatment of retinopathy of prematurity: results of the early treatment for retinopathy of prematurity randomized trial. *Arch Ophthalmol*. 2003;121:1684–1694.
13. International Committee for the Classification of Retinopathy of Prematurity. The international classification of retinopathy of prematurity revisited. *Arch Ophthalmol*. 2005;123:991–999.
14. Repka MX, Palmer EA, Tung B. Involution of retinopathy of prematurity. Cryotherapy for Retinopathy of Prematurity Cooperative Group. *Arch Ophthalmol*. 2000;118:645–649.
15. Wetherill GB, Levitt H. Sequential estimation of points on a psychometric function. *Br J Math Stat Psychol*. 1965;18:1–10.
16. Barlow HB. Increment thresholds at low intensities considered as signal/noise discriminations. *J Physiol*. 1957;136:469–488.
17. Baylor DA, Nunn BJ, Schnapf JL. The photocurrent, noise and spectral sensitivity of rods of the monkey Macaca fascicularis. *J Physiol*. 1984;357:575–607.
18. Dunn FA, Rieke F. The impact of photoreceptor noise on retinal gain controls. *Curr Opin Neurobiol*. 2006;16:363–370.
19. Brown AM. Scotopic sensitivity of the two-month-old human infant. *Vision Res*. 1986;26:707–710.

20. Sharpe LT, Stockman A, Fach CC, Markstahler U. Temporal and spatial summation in the human rod visual system. *J Physiol*. 1993;463:325–348.
21. Hansen RM, Moskowitz A, Tavormina JL, Bush JN, Soni G, Fulton AB. Temporal summation in children with a history of retinopathy of prematurity. *Invest Ophthalmol Vis Sci*. 2015; 56:914–917.
22. Lamb TD. Dark adaptation: a re-examination. In: Hess RF, Sharpe LT, Nordby K, eds. *Night Vision: Basic, Clinical and Applied Aspects*. Cambridge: Cambridge University Press; 1990:177–222.
23. Lamb TD, Pugh EN Jr. Phototransduction, dark adaptation, and rhodopsin regeneration the proctor lecture. *Invest Ophthalmol Vis Sci*. 2006;47:5137–5152.
24. Fulton AB, Reynaud X, Hansen RM, Lemere CA, Parker C, Williams TP. Rod photoreceptors in infant rats with a history of oxygen exposure. *Invest Ophthalmol Vis Sci*. 1999;40:168–174.
25. Angueyra JM, Rieke F. Origin and effect of phototransduction noise in primate cone photoreceptors. *Nat Neurosci*. 2013;16: 1692–1700.
26. Donner K. Noise and the absolute thresholds of cone and rod vision. *Vision Res*. 1992;32:853–866.

Investigative Ophthalmology & Visual Science