P23H Rhodopsin Transgenic Rat: Correlation of Retinal Function with Histopathology

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Among the more than 80 different rhodopsin mutations associated with autosomal dominant retinitis pigmentosa (adRP), P23H was the first and accounts for approximately 12% of adRP families of US origin. The mechanism by which the P23H mutation leads to photoreceptor cell death remains unknown, although faulty trafficking and defective disc morphogenesis have been suggested. Laboratory studies to rescue photoreceptor cells from degeneration use animal models that mimic human RP. Strategies to slow photoreceptor degeneration include survival-promoting factors, retinal transplantation, and gene therapy. P23H transgenic mice, including the VPP mouse, have been developed and characterized for retinal histopathology and function, and these murine models manifest progressive retinal degeneration similar to that in human adRP. However, the tiny mouse eye poses difficulty with surgical manipulations, and this led to the development of the rhodopsin transgenic rat, which has larger eyes. The P23H transgenic rat carries a mutant mouse opsin gene in addition to the endogenous native opsin genes and undergoes a gradual photoreceptor loss that is generally characteristic of human adRP.

To use the P23H rhodopsin transgenic rat for rescue studies, we wanted a careful description of the natural history of degeneration against which to judge therapeutic success. The electroretinogram (ERG) provides an important means of tracking degeneration noninvasively; and in these studies, we particularly wanted to correlate ERG changes with the histopathology. Results showed that this P23H transgenic rat has slowly progressive rod dysfunction, with initially normal cone function, consistent in broad outline with clinical findings reported in human P23H adRP patients. However, the P23H rat showed some functional differences from human disease in the results of phototransduction modeling and the presence of a normal rate of recovery from bleach. We found that the a-wave was more sensitive than the b-wave for tracking the histopathologic status across the wide range of photoreceptor cell loss in this rat RP model.
METHODS

Animals

These studies were conducted in accordance with the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research. P23H homozygous animals for breeding were kindly provided by Matthew LaVail, PhD (UCSF School of Medicine, Beckman Vision Center), and were bred in our laboratory against albino Sprague-Dawley (SD) rats (Harlan, Indianapolis, IN) to produce P23H heterozygous rats that have a single P23H transgene allele in addition to the normal two wild-type opsin alleles. Lines from two different original founder animals were studied: Line 1 animals have a higher level of transgene expression than line 3 and degenerate faster than line 3. Lines 1 and 3 were both studied, because they may provide information about late severe and early mild degeneration, respectively. Approximately 100 transgenic P23H rats and 30 normal SD albino control animals from the same breeding stock were used in this study; across ages 4 to 29 weeks. All rats of both types were bred, born, and reared in our laboratory and were fed high-fat breeding chow (Formulab; PMI Feed, Richmond, IN) ad libitum. All rats were kept on a 12/12-hour light/dark cycle for corneal hydration. A chloride silver reference electrode was simultaneously using chlorided silver wire loops on the corneas, with 1% tetracaine topical anesthesia and a drop of methylcellulose for corneal hydration. A chloride silver reference electrode was placed on the sclera 1 mm from the temporal limbus of each eye. The ground electrode was on the left ear. Responses were amplified at 10,000 gain at 0.1 to 1000 Hz, filtered to remove 60 Hz noise, and digitized at a 20-kHz rate. Bright flash ERG responses were amplified to 60 seconds depending on intensity, and 20 to 30 photopic responses were computer-averaged with stimulus intervals of 3 sec flashes of 3.4 log cd-s/m² (model PS33; Grass Instruments, Quincy, MA). This flash intensity is quite dim and elicits a rod-isolated b-wave with essentially no contribution of the cones17; flashes every 5 minutes do not desensitize the response. Responses during recovery were normalized by the dark-adapted b-wave amplitude to this same stimulus recorded immediately before the bleach.

Phototransduction Modeling of Saturated a-Waves

Bright flash ERG a-waves were recorded from both eyes simultaneously with gold wire electrodes to avoid photovoltaic artifacts. Responses were amplified at 5000 gain from 1 to 1000 Hz and digitized at 20 kHz. Bright 1-msec photostrobe flashes (model 283; Vivitar, Santa Monica, CA; color temperature 5500°K) were presented in a Ganzfeld Bowl, with maximal intensity of 2.1 log cd-s/m², which produced a maximal retinal intensity of 3.57 log scotopic troland (td-s) and attenuated by neutral density filters with 0.5 log unit steps. Flashes were spaced at 1 to 15 minutes to avoid adapting the a-wave. Bright flash ERGs were recorded from 4-week-old rats using 5 each of P23H lines 1 and 3 and SD controls.

The leading edge of the a-wave (P3) was fitted with the Hood and Birch version18 of the Lamb and Pugh model19 (see Eq. 1 below), with a-wave termination chosen as just before the upturn of the a-wave or 15 msec after flash onset. The effective time delay (T_{eff}) between flash and a-wave onset was determined by fitting a straight line to the baseline data, and to 1 msec of data after the signal deviates three standard deviations beyond the baseline noise20 at the highest intensity. T_{eff} is set as the point where the two lines intersect and is fixed for all lesser intensities. To estimate T_{eff} independent of observer bias, R_{max} was set equal to the value of the a-wave elicited by the brightest flash intensity. The sensitivity (S) was the only parameter allowed to vary and was fit to individual traces to observe the changes in S with intensity.

\[
P_{3}(I, T) = R_{max} \frac{1}{1 - \exp(-\frac{1}{2} I \cdot S \cdot (T - T_{eff})^{2})} \tag{1}
\]

where I represents stimulus intensity and T represents time.

Histology

Rats were killed with sodium pentobarbital overdose 1 day after ERG recordings. Eyes were enucleated and fixed overnight at 4°C in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Eyes were postfixed in 1% osmium tetroxide for 1 hour. Epon-embedded tissue was sectioned 1-μm thick along the vertical meridian through the optic nerve and stained with toluidine blue for light microscopy. Column cell counts of outer nuclear layer (ONL) thickness and the length of the rod outer segment (ROS) were determined every 400 μm across both the superior and inferior hemispheres, and the resulting numbers were averaged for each retina to obtain a measure of cellular changes across the entire retina. This provided a whole-retina measurement for comparison with Ganzfeld ERG function.

Statistical Analysis

The two-tailed Student’s t-test was used to compare paired data from age-matched control and transgenic rats. Data across ages were analyzed with the Kruskal-Wallis test, which is a form of ANOVA that does not presume any specific distribution.

RESULTS

Histology

Line 1 exhibits faster degeneration than line 3, and by 4 weeks of age the line 1 ROS was 40% shortened and the ONL had...
approximately 40% loss of cells compared with controls (Figs. 1 and 2). By 29 weeks of age the ROS of line 1 was severely shortened to less than 12% of control, and the ONL had fewer than two rows of cells remaining, or 18% of control width. At ages 4 to 29 weeks in line 1 and ages 8 to 29 weeks in line 3, the ROS length and ONL width were significantly reduced from controls ($P < 0.01$; Fig. 2); for line 3 at 4 weeks of age, ROS length was not significantly different from control ($P = 0.30$). By 29 weeks of age in line 3, the ONL and ROS were both about one-half control thickness. For therapy considerations we wanted to know the change in each line over the period of 4 to 29 weeks; at 29 weeks of age, ONL cell count was reduced to 27% of the 4-week-old baseline in line 1 and to 52% in line 3. The ONL appeared to thin more rapidly between 4 and 8 weeks in both lines and then decline more slowly.

The effect of superior versus inferior hemisphere on ONL width by cell count was analyzed for line 3 across ages 4 to 29 weeks and was not significant by ANOVA ($P = 0.44$). Similar analysis for line 1 also indicated no significant difference of ONL width in the two hemispheres ($P = 0.27$).

ROS length was correlated with ONL width across both lines 1 and 3 ($R = 0.93$, Fig. 2B). In line 1 at 29 weeks of age, ROS length deviated downward from the regression line because end-stage disease had thinned the ONL to less than a two cell width. This suggests that photoreceptor cells can survive for a short time even with very little ROS. An example of this is found in the rhodopsin knockout mouse, which initially produces a full complement of rod cells that fail to elaborate ROS but survive for many weeks before ultimately dying by 11 weeks.

**Scotopic ERG**

The dark-adapted ERG was characterized by three measures: threshold for criterion amplitude responses, rod-mediated scotopic maximum amplitude ($V_{\text{max}}$), and intensity to elicit one-half maximum amplitude (also termed the “k” value, or sensitivity) (Fig. 3 and Table 1). For dark-adapted recordings, the maximum rod b-wave ($V_{\text{bmax}}$) without cone contamination occurs just before the dip of the intensity-response function. This dip is easily seen in line 3 animals but is difficult to judge in line 1 rats because of an a-wave amplitude loss, and $V_{\text{bmax}}$ was taken as the b-wave amplitude on the plateau just before the second increase. $V_{\text{bmax}}$ was significantly smaller for the transgenic rats, except for 4-week-old line 3 animals, compared with SD controls ($P < 0.01$) and decreased progressively with age in both lines ($P < 0.01$). In line 1 $V_{\text{bmax}}$ decreased rapidly between 15 and 29 weeks, as the ONL is thinned to below 2 cell width.

The rat has a minimal cone a-wave, and the dark-adapted a-wave plots in Figure 3 reflect rod activity nearly exclusively. For transgenic rats the dark-adapted rod a-wave maximum amplitudes ($V_{\text{amax}}$) were significantly smaller than for SD controls ($P < 0.01$) and decreased progressively with age ($P < 0.01$ for both lines). Although responses of line 1 were smaller than line 3, the relative $V_{\text{amax}}$ loss over the period 4 to 29 weeks was similar for line 1 (80%) and line 3 (74%), and by this criterion both lines were changing at similar relative rates. Because a-wave thresholds increased with age, it was surprising that b-wave thresholds remained virtually constant within each line for all but the end-stage condition of line 1 at 29 weeks (Fig. 3). At 4 weeks of age, b-wave threshold was significantly elevated for both line 1 and line 3 compared with the SD controls ($P < 0.01$). However, thereafter for each line, thresholds changed minimally with age for either line (0.15 log unit for line 3 at 4 to 29 weeks, and 0.10 log unit for line 1 at 4 to 15 weeks) and were comparable to the threshold variation with aging found for the SD controls (0.08 log unit, 4 to 29 weeks). Testing for non-zero slope by linear regression of
b-wave threshold across ages 4 to 29 weeks gave $P = 0.12$ for all animals in line 1 and $P = 0.21$ for all animals in line 3, indicating that threshold was essentially static with age. This suggests that some compensatory mechanism was acting on the b-wave to stabilize threshold despite the progressive photoreceptor loss. The sole exception was in line 1, in which the threshold increased by 0.3 log unit between 15 to 29 weeks; these advanced animals had massive loss of rod cells and ROS shortening by this age and showed an overall shift in the V-log I response function (Fig. 3C).

Correlations between Retinal Function and Histopathology

$V_{a_{\text{max}}}$ and $V_{b_{\text{max}}}$ are frequently used to correlate with ONL cell counts as an indication of the stage of retinal degeneration. Figure 4 shows data from lines 1 and 3 plotted together. The change in $\log V_{\text{max}}$ of both the a- and b-waves was proportional to ONL cell loss (a-wave, $R = 0.94$; b-wave, $R = 0.89$; Fig. 4), but $V_{a_{\text{max}}}$ slope was approximately double that of $V_{b_{\text{max}}}$, indicating that $V_{a_{\text{max}}}$ is more sensitive for detecting photoreceptor cell loss. Note that in end-stage disease the lowest two points deviate downward from these straight lines, as expected, because when all cells are lost, both $V_{a_{\text{max}}}$ and $V_{b_{\text{max}}}$ must head toward “zero.” What seems remarkable is the good fit of these regression lines across data pooled from both lines 1 and 3 comprising a more than 80% range of cell loss. This suggests that the degeneration process is similar across these two lines for all but end-stage ONL cell loss.

The a-wave threshold also showed a log increase with ONL cell loss ($R = 0.93$, not shown). As described for Figure 3, b-wave threshold remained essentially static with age within.

**Figure 2.** (A) Averages across the entire retina vertical meridian of ONL cell counts and ROS length in SD control and P23H rhodopsin transgenic line 1 and line 3 rats at ages of 4 to 29 weeks. SE bars are shown. Compared with age-matched controls, the differences were significant ($^* P < 0.01$) for both lines, except for ROS length in line 3 at 4 weeks of age. (B) ROS length versus ONL cell counts for individual retinas of lines 1 and 3.

**Figure 3.** Scotopic ERG intensity–response ($\log V - \log I$) function of a- and b-waves in the SD control (A) and P23H rhodopsin transgenic line 3 (B) and line 1 (C) rats. Criterion amplitudes: a-wave 20 $\mu$V and b-wave 50 $\mu$V. SE bars are shown.
each of lines 1 and 3, and by threshold measurements, the a-wave was more sensitive to the b-wave for tracking cell loss.

In a previous study, saturated amplitude of fast PII was proportional to ROS length for albino rats reared under a different lighting intensity.23 In that study, ROS length was manipulated through the process of photostasis to two light-rearing intensities. The P23H rhodopsin mutant animals provide an opportunity to extend this observation to include the case of cell loss in addition to ROS shortening. A-wave saturation occurs at the flash intensity sufficient to close all cGMP-gated channels and to completely interrupt the rod dark-current. If one assumes that channel density per unit area of ROS plasma membrane remains constant despite a change in ROS length, then the total dark-current per rod would be proportional to ROS length, as found by Reiser et al.23 However, in the P23H rat, total retinal photocurrent will also be proportional to the number of rod cells remaining. Consequently, for the P23H rat, the maximum photocurrent change on complete channel closure with a saturating ERG flash would be proportional to the product of ROS length and ONL cell count (ROS × ONL). Figure 5A provides support for this idea by the tight linear correlation between Va\textsubscript{max} and ROS × ONL across both lines 1 and 3 (R = 0.96). Note that, like the Ganzfeld ERG, the histology represents a global retinal assessment, because ROS and ONL were averaged across the entire retina. These data are well fitted across a 100-fold range for both Va\textsubscript{max} and ROS × ONL, and the line regresses to zero a-wave voltage for zero ROS × ONL. This suggests that ROS plasma membrane channel density is maintained during degeneration. Neither ROS nor ONL alone gives a direct relationship of Va\textsubscript{max}. ONL is related to the logarithm of Va\textsubscript{max} (Fig. 4) and consequently deviates considerably from direct proportionality. The same is true for ROS versus Va\textsubscript{max} (not shown but as one might anticipate from the linear relationship of ROS with ONL shown in Fig. 2B).

Figure 5B shows that b-wave amplitude is linearly correlated with the a-wave (R = 0.95), and tracking the b-wave may provide a substitute for a-wave recordings, particularly for higher amplitude responses. The tight correlation indicates linearity of visual signal transfer across the rod to bipolar synapse. However, this relationship fails for end-stage disease when the a-wave is greatly reduced, and the b-wave persists even when the a-wave voltage approaches zero. The b-wave offset from zero must be interpreted carefully, because this cannot be taken to imply that rod signals reach the proximal retina despite a complete loss of rods. This may reflect signal divergence from the very few rods that remain and signal amplification by the second and third order neurons.24 Alternatively, signals might derive from remaining cone cells, because cones contribute very little to the a-wave. In any case, the b-wave amplitude appears to be a more complex and consequently less direct measure of residual rod function in end-stage disease.

![Figure 4](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932909/)

**FIGURE 4.** Correlation of ONL cell counts with maximum amplitude of the scotopic a- and b-wave in P23H transgenic rats.

### Table 1. Scotopic ERG Changes with Age

<table>
<thead>
<tr>
<th></th>
<th>a-Wave</th>
<th>b-Wave</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Va\textsubscript{max}† (log (\mu V))</td>
<td>Threshold‡ (log (cd/m^2))</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 W, n = 5</td>
<td>2.79 ± 0.05</td>
<td>-0.60 ± 0.14</td>
</tr>
<tr>
<td>8 W, n = 5</td>
<td>2.75 ± 0.02</td>
<td>-0.53 ± 0.17</td>
</tr>
<tr>
<td>15 W, n = 5</td>
<td>2.59 ± 0.05</td>
<td>-0.53 ± 0.09</td>
</tr>
<tr>
<td>29 W, n = 3</td>
<td>2.56 ± 0.06</td>
<td>-0.30 ± 0.26</td>
</tr>
<tr>
<td>Line 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 W, n = 5</td>
<td>2.20 ± 0.04**</td>
<td>0.31 ± 0.19**</td>
</tr>
<tr>
<td>8 W, n = 5</td>
<td>2.02 ± 0.10**</td>
<td>0.39 ± 0.18**</td>
</tr>
<tr>
<td>15 W, n = 4</td>
<td>1.81 ± 0.04**</td>
<td>0.74 ± 0.11**</td>
</tr>
<tr>
<td>29 W, n = 3</td>
<td>1.45 ± 0.22**</td>
<td>1.16 ± 0.06</td>
</tr>
<tr>
<td>Line 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 W, n = 6</td>
<td>2.67 ± 0.06**</td>
<td>-0.44 ± 0.14</td>
</tr>
<tr>
<td>8 W, n = 5</td>
<td>2.48 ± 0.05**</td>
<td>-0.02 ± 0.11**</td>
</tr>
<tr>
<td>15 W, n = 4</td>
<td>2.39 ± 0.05**</td>
<td>0.51 ± 0.02**</td>
</tr>
<tr>
<td>29 W, n = 3</td>
<td>2.09 ± 0.06**</td>
<td>0.50 ± 0.04**</td>
</tr>
</tbody>
</table>

† Stimulus intensity: 2.2 log \(cd/m^2\); ‡ criterion amplitude: 20 \(\mu V\) for the a-wave and 50 \(\mu V\) for the b-wave.

* P < 0.05; ** P < 0.01. Tabulated values give mean ± SD.
Phototransduction a-Wave Modeling

Figure 6A shows ERG responses to a bright flash, averaged from 5 animals of line 1 and 5 SD controls at 4 weeks of age. The a-wave amplitude of the P23H rat line 1 was approximately 25% of the SD control at 4 weeks of age, but in this figure it was normalized to the SD control a-wave maximum. The b-wave amplitude relative to the a-wave was considerably larger for the transgenic rats compared with SD controls \((P < 0.01)\), which is consistent with the evidence shown in Figure 3 that the b-wave amplitude was more preserved than the a-wave in this model. The peak implicit time of the transgenic a-wave was approximately 1 msec faster than the control (Fig. 6A, inset), indicating greater sensitivity for the P23H response. The same observation was made by formal fitting of the a-wave with the phototransduction model (see Eq. 1). Values of \(S\) were averaged across five animals in each group and were plotted as a function of stimulus intensity (Fig. 6B). \(R_{\text{max}}\) of the a-wave was reduced to 25% and 88% of SD controls for lines 1 and 3, respectively, at 4 weeks, roughly proportional to ROS ONL, which were 35% and 77% of the control value for these lines at this age. The log \(S\) values for transgenic rats was higher across the entire stimulus intensity range compared with SD control rats despite the considerable \(R_{\text{max}}\) reduction.

Recovery after Rhodopsin Bleach

Recovery of retinal function after bleaching rhodopsin was studied in 8-week-old line 3 animals (Fig. 7), at which time the ONL thickness was approximately 70% of the controls (Fig. 2A). The stimulus intensity was sufficiently dim to avoid cone contributions. b-Wave recovery reflects network adaptation early in the course, but after a short period (approximately 10 minutes in skate) it tracks the receptor potential and recovers in parallel with rhodopsin regeneration. The time course of recovery of the P23H rats tracked well with SD controls across 90 minutes, with complete overlap of the SE bars at all time points. The greatest deviation of P23H from control was at approximately 60 minutes, but the normalized recovery amplitude was not different from SD controls \((P = 0.70, \text{at 60 minutes})\).

Photopic ERG

Photopic cone-driven ERG b-waves of line 3 rats showed no difference \((P > 0.05)\) from SD controls for amplitude and threshold at any age (4 to 29 weeks; Fig. 8). For line 1, cone b-wave amplitude and threshold initially were comparable to SD controls but progressively deteriorated with age and were significantly decreased at 8 to 29 weeks \((P < 0.01)\).
of age in line 1, more than half of the rod photoreceptors were already lost (Fig. 2A).

In both the transgenic and SD control animals, the photopic b-wave amplitude underwent a major decrease between 4 and 8 weeks. Because controls also showed the amplitude drop, this is unlikely due to cone cell death and suggests that some shift occurs in the cell populations that contribute to or affect the photopic b-wave. This could reflect maturation of proximal retinal activity that contributes a negative component under photopic conditions and that is exaggerated in some forms of rat retinal degeneration.28

DISCUSSION

Comparison of the P23H Transgenic Rat with Human P23H adRP

The P23H rat retinal degeneration grossly mimicked the human adRP in several aspects. Abnormal rod ERG function was detected as early as 4 weeks of age (Table 1) in both transgenic lines, which indicates an early onset of retinal degeneration in this rat model. Although line 3 at 4 weeks showed minimal change in histology, with only a 15% reduction of ONL cell counts and slightly shortened ROS, the a-wave amplitude was already significantly reduced. ERG impairment is an early clinical manifestation in adRP patients even without subjective symptoms.3,29

Line 3 degenerated comparatively slowly out to 29 weeks, as assessed by histology and the ERG. Even in line 1, with massive cell loss by one-half year age, these animals retained more than 20% of dark-adapted b-wave amplitude at 29 weeks of age. Rats usually do not live beyond 2 years, and this functional retention at one-half year suggests that these animals retain vision for a relatively long period of their lives. P23H patients have significantly better visual acuity and larger ERG amplitudes than adRP from other mutations and may retain useful vision to approximately 70 years of age.3

ERG rod function was impaired before photopic cone function, which is consistent with clinical P23H patients.3 The photopic ERG in line 1 rats was normal at 4 weeks and began to decline by 8 weeks of age, when the ONL was thinned by one half, suggesting that loss of considerable number of rods can occur before significant cone degeneration in this rat model. Regional distribution of retinal degeneration is seen in human P23H disease,29,30 probably associated at least in part with geographic differences of the relative rod/cone ratio in the human retina. In the P23H rat the degeneration is relatively uniform across the retina, and no significant difference was found between superior and inferior hemispheres in remaining ONL cells at any age of 4 to 29 weeks of age.

The rod b-wave of the P23H rat recovered normally after a bleaching light exposure, unlike human P23H disease in which dark-adaptation is markedly slowed by as much as twice normal.31 This is also dissimilar to the VPP mouse with a P23H rhodopsin mutation which shows slow rod b-wave recovery from bleach.12 P23H opsin is partially misfolded but correctly forms the pocket for 11-cis-retinal.32 The ERG data in the P23H rat indicates that reconstituting of rhodopsin after bleach appears to be normal.

P23H rat also differs from human P23H disease in transduction sensitivity. The P23H rats showed no decrease in phototransduction sensitivity, and this was also normal in the...
VPP mice with a P23H mutation. However, reduced sensitivity was reported for human P23H disease, with 5 decreased by 0.5 to 0.9 log unit in three patients, including two young individuals of 18 and 27 years of age who retained good visual field diameter. These patients had severely impaired rod and cone function; however, with $K_v$ (rod b-wave semisaturation constant) elevated by more than one log unit, which is considerably more than the 0.4 log unit elevation of even our most affected P23H line 1 rats at the 29-week age. Consequently, one may suspect that these patients had advanced retinal histopathologic changes that contributed to the decreased phototransduction sensitivity rather than resulting from molecular changes of opsin.

**ERG Changes in P23H Rat Degeneration**

Sensitivity of the a-wave (determined as the intensity for one-half $V_{a_{max}}$) of SD controls and line 3 animals at all ages was approximately 1.0 log cd/m² (Table 1). Even at 29 weeks of age when the line 3 animals produced a-waves only one half the amplitude of controls and had histologic ROS shortening and approximately 50% ONL cell loss, the a-wave sensitivity was approximately 0.1 log unit different from that of controls. Similarly, for line 1 animals with severe degeneration and only 10% of normal a-wave amplitude by 29 weeks, very little change of a-wave sensitivity was found; the change was less than 0.2 log unit at 29 weeks compared with both 4-week-old line 1 animals and 29-week-old control animals. This indicates that the input–output relationship of the rod cell in converting incident light to ROS membrane photovoltage/photocurrent change remains essentially constant despite degeneration. This is further confirmation of the findings from formal phototransduction a-wave modeling, in which log 3 values were preserved in line 1 despite a 75% a-wave amplitude loss.

The b-wave was preserved relatively better than the a-wave as the retinal degeneration progressed. This suggests that enhanced gain may occur at the photoreceptors or beyond to compensate for decreasing photic input as the ROS shortens and the rod cell counts decrease during degeneration. However, there appears to be a limit to the compensation, because the scotopic maximum b-wave amplitude was considerably decreased by 29 weeks in P23H line 1 rats with advanced degeneration to 2 ONL rows and less.

The mechanism of this phenomenon remains uncertain. One possible explanation is that the wide receptive field of the bipolar cell provides buffering against loss of photoreceptors. Bipolar cells receive input from as many as 45 rods, and a maximal bipolar response might result from signaling by fewer than all these rods. In such case, the maximal b-wave response could be reached in degeneration even though the pooled a-wave response of rod photocurrents was decreased. However, with very advanced degeneration, the rod bipolar cell may lose input from enough rods to cause the scotopic b-wave amplitude to decline.

A second possibility is that synaptic plasticity may allow some degree of compensation of retinal signaling as rods are lost. In the peripheral nervous system, loss of presynaptic elements from one motor neuron during development causes an increase in signaling of the remaining elements. Synaptic rearrangement may also take place in the adult central nervous system, and growth factors such as transforming growth factor-β can strengthen synaptic signaling. Furthermore, in the retina the rod-to-bipolar cell synapse shows signaling plasticity during diurnal light cycling of the day/night rhythm. This suggests that synaptic remodeling could occur in the P23H rat retina during photoreceptor degeneration. Evidence of this would require electron microscopy rather than ERG methodology.

The scotopic b-wave near its threshold was surprisingly preserved and remained nearly constant in each line throughout the 29-week study, except for the 29-week line 1 rats with quite advanced ONL and ROS degeneration. If dimmer stimuli activate rhodopsin and close cGMP-gated channels preferentially at the base rather than the tip of ROS, b-wave threshold might be relatively protected against modest ROS shortening.

The RCS rat also shows relative preservation of postphotoreceptoral ERG responses during retinal degeneration, particularly for the scotopic threshold response (STR), which reflects amacrine cell activity. Relative sparing of STR, despite progressive loss of photoreceptors, is also reported in light-damaged rats. This is similar to the P23H rat, in which the oscillatory potentials (OPs) in Figure 6A were well preserved for these line 1 animals with a 75% reduction of the a-wave. Although we did not systematically analyze the OPs in this study, this suggests that other postphotoreceptoral potentials, in addition to the b-wave, are preserved relatively better than the a-wave at this stage of P23H disease.

**Correlation between the ERG and Histopathology**

This study sought to identify ERG parameters that were sensitive to the extent of degeneration, for use in studying efficacy of photoreceptor rescue strategies. The saturated a-wave amplitude correlated directly with the product of (ROS × ONL) across nearly two log units of a-wave amplitude and cellular structural changes, in data pooled across lines 1 and 3. The product (ROS × ONL) should reflect the total remaining ROS plasma membrane area, which contains the cGMP-gated channel. This extends the observations of Reiser et al. by including cell loss in addition to a wide range of ROS shortening and adds to the conclusion that the saturated a-wave amplitude directly reflects rod photocurrent. Both the a-wave amplitude and the photoreceptor structural change and loss regressed to zero simultaneously. Consequently, the a-wave provides an excellent noninvasive measure for tracking progression of photoreceptor disease in these P23H rats. If the correlation of a-wave with (ROS × ONL) is borne out in other animal models of retinal degeneration, this may provide a useful measure to judge the extent of rod degeneration in human RP disease.

a-Wave modeling gave normal phototransduction sensitivity for transgenic P23H rats despite ROS shortening and the outright loss of photoreceptors. Furthermore, the tight linear correlation of maximum a-wave amplitude with (ROS × ONL) indicates that plasma membrane density of cGMP-gated channels seems to remain constant despite ROS shortening. These findings suggest that the remaining photoreceptors in the P23H rat retain good function despite having short ROSs. b-Wave maximum amplitude and threshold were both preserved better than those of the a-wave in advanced disease, indicating that a-wave measures are preferable for tracking degeneration across the entire disease spectrum.

The P23H rat ERG was slightly different from light damage rat for which a log–linear relationship was found between ONL and ROS degeneration. Evidence of this would require electron microscopy rather than ERG methodology.
becomes quite large and reduces the b-wave amplitude and impairs b-wave threshold. The P23H rat does not have an exaggerated STR (data not shown).

**SUMMARY**

The P23H rhodopsin transgenic rat has a comparatively slow rod degeneration with initial sparing of cone function. Although this is similar to human P23H adRP, differences were noted in the rate of recovery from a light bleach and in transduction sensitivity from a-wave modeling. Furthermore, the remarkable relative preservation of b-wave threshold. The P23H rat does not have an exaggerated STR (data not shown).

**Acknowledgments**

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**References**