The Status of Cones in the Rhodopsin Mutant P23H-3 Retina: Light-Regulated Damage and Repair in Parallel with Rods

Vicki Chrysostomou,1 Jonathan Stone,1 Sally Stowe,1 Nigel L. Barnett,2 and Krisztina Valter1

PURPOSE. This study tests whether cones in the rhodopsin-mutant transgenic P23H-3 retina are damaged by ambient light and whether subsequent light restriction allows repair of damaged cones.

METHODS. P23H-3 rats were raised in scotopic cyclic (12 hours of 5 lux, 12 hours of dark) ambient light. At postnatal day 90 to 130, some were transferred to photopic conditions (12 hours of 300 lux, 12 hours of dark) for 1 week and then returned to scotopic conditions for up to 5 weeks. Photoreceptor function was assessed by the dark-adapted flash-evoked electroretinogram, using a two-flash paradigm to isolate the cone response. Outer-segment structure was demonstrated by immunohistochemistry for cone and rod opsins and by electron microscopy.

RESULTS. Exposure for 1 week to photopic ambient light reduced the cone b-wave, the rod b-wave, and the rod a-wave by 40% to 60% and caused shortening and disorganization of cone and rod outer segments. Restoration of scotopic conditions for 2 to 5 weeks allowed partial recovery of the cone b-wave and the rod a- and b-waves, and regrowth of outer segments.

CONCLUSIONS. Modest increases in ambient light cause rapid and significantly reversible loss of cone and rod function in the P23H-3 retina. The reduction and recovery of cone function are associated with shortening and regrowths of outer segments. Because the P23H mutation affects a protein expressed specifically in rods, this study emphasizes the close dependence of cones on rod function. It also demonstrates the capacity of cones and rods to repair their structure and regain function.

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Experimental Design

P23H-3 animals were raised in cyclic ambient light (12 hours of light, 12 hours of dark) with the light phase at 5 lux (scotopic conditions), to postnatal day 90 to 130. Animals were moved to photopic ambient conditions (12 hours of 300 lux, 12 hours of dark) at the end of the 1 week exposure, and 2 and 5 weeks after exposure. The electrotretinogram (ERG) was recorded serially from seven animals at each of the above four time points. Tissue was collected from an additional five animals at each time point. Tissue from scotopic-reared SD rats was processed for comparison.

Tissue Collection and Processing

Animals were euthanatized with an overdose of pentobarbital sodium (>60 mg/kg, IP). Eyes were marked with an insoluble projection pen at the superior aspect of the limbus for orientation, enucleated, and immersion-fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) at pH 7.4 for 5 hours. The left eye of each animal was processed for cryosectioning, and the right eye was processed for wholemounts or electron microscopy. For cryosectioning, the eyes were rinsed twice in 0.1 M PBS and left in a 15% sucrose solution overnight to provide cryoprotection. The eyes were embedded in OCT compound (Tissue-Tek; Sakura Finetek, Tokyo, Japan), and snap frozen in liquid nitrogen before taking 12 μm cryosections at −20 °C (CM1850 Cryostat; Leica, Wetzlar, Germany). Sections were mounted on gelatin and poly-L-lysine-coated slides and dried overnight at 50 °C before being stored at −20 °C. For retinal wholemounts, the retina was dissected from the eye cup, flattened by making radial incisions, gently sandwiched between two glass slides and immersed in 4% paraformaldehyde at 4 °C for up to 2 weeks before labeling.

Outer Segment Status

**Immunohistochemistry of Sections.** Cryosections were labeled with rabbit polyclonal antibodies to L/M opsin (1:1000; Chemicon, Temecula, CA) and mouse monoclonal antibodies to rod opsin (1:1100, Rho4D2, a gift from Robert Molday, University of British Columbia, Vancouver, BC, Canada). Sections were washed in 70% ethanol for 15 minutes, followed by a 5-minute wash in distilled H2O and two 5-minute washes in 0.1 M PBS. Sections were then blocked with 10% normal goat serum in 0.1 M phosphate-buffered saline (PBS) at pH 7.4 for 1 hour before being incubated for 24 hours at 4 °C with a mixture of the above primary antibodies. After two 10-minute washes in 0.1 M PBS, sections were treated with an antibody to rabbit IgG conjugated with Alexa Fluor 488 and with an antibody to mouse IgG conjugated with Alexa Fluor 594 (1:1000; Invitrogen-Molecular Probes, Eugene, OR) for 24 hours at 4 °C. After they were washed twice in 0.1 M PBS, the sections were incubated for 2 minutes with the DNP-specific dye bisbenzimidine (10:1000) in 0.1 M PBS before being coverslipped with a glycerol-gelatin medium.

Measurements of cone outer segment (OS) length were made on digital images of cryosections immunolabeled with L/M opsin. Sections were scanned from the superior to inferior edge of the retina and, at regularly spaced intervals, the length of L/M opsin-stained OSs was measured (a total of at least 24 measurements per retina). Results from five animals at each time point were averaged and analyzed by the statistical method described in the next section.

**Immunohistochemistry of Wholemounts.** Retinas were dehydrated in ascending ethanol, incubated in 100% ethanol for 24 hours at 4 °C and then rehydrated. The tissue was rinsed twice in 0.01% Triton-PBS and blocked in normal goat serum for 2 hours before incubation with rabbit polyclonal antibodies to L/M opsin (1:1000, Chemicon) for 24 hours. After they were washed in 0.01% Triton-PBS, retinas were incubated for 24 hours with an antibody to rabbit IgG conjugated to Alexa Fluor 488 (1:1000; Invitrogen-Molecular Probes) and subsequently mounted and coverslipped on glass slides with the photoreceptor OSs facing up. To measure L/M cone density, we photographed the OS layer with a 10× objective, systematically reconstructing the whole surface. This method required ~200 separate digital images, spliced into a full montage. Cone OS density was then measured over areas of 0.01 mm², spaced at 0.5-mm intervals across the whole surface.

**Electron Microscopy.** After removal, eyes were immersed in cold fixative (2.5% glutaraldehyde plus 5% formaldehyde in 0.1 M sodium cacodylate buffer [pH 7.4], with 5 mM EGTA and 2 mM MgCl2), and the lenses were removed rapidly. Within 10 to 15 minutes, the eyes were microwaved on ice using six 10-second bursts at 10-second intervals, at 80 mW in a histologic microwave oven (Pelco BioWave; Ted Pella, Inc., Redding, CA). After 2 to 4 hours' further fixation at 4 °C, the anterior portions of the eyes were removed, and selected retinal pieces were dissected. After it was washed in cacodylate buffer and postfixed in 1% osmium tetroxide in the same buffer, the tissue was dehydrated through ethanol and acetone and embedded in Epon-Araldite. Sections were cut at 50 to 80 nm (Ultructra E; Leica), stained with uranyl acetate alone or followed by Reynolds' lead citrate, and viewed at 75 kV in a transmission electron microscope (TEM, H7100; Hitachi). Sections (0.5 μm) from the same blocks were stained with toluidine blue for light microscopy.

**Retinal Thickness Measurements**

Measurements were made on digital images of cryosections stained with bisbenzimidine. At each measurement location, the thickness of the outer nuclear layer (ONL) as well as the thickness of the retina, from inner to outer limiting membrane (ILM-OLM), was recorded. The ratio of ONL to ILM-OLM was used for analysis to account for obliquely cut sections. In at least two sections per animal, we took four measurements, approximately 100 μm apart, from both the superior and inferior midperipheral areas of the retina (a total of at least 16 measurements per animal). Results from five animals at each time point were averaged and analyzed by the statistical method described below.

**Electrotretinography**

The function of photoreceptors was assessed from the flash-evoked ERG, as described previously. Animals were dark-adapted overnight and prepared in dim red illumination. We used a two-flash paradigm to isolate the cone and (by subtraction) the rod responses of the ERG. To construct the whole surface. This method required ~200 separate digital images, spliced into a full montage. Cone OS density was then measured over areas of 0.01 mm², spaced at 0.5-mm intervals across the whole surface.

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We used three measurements of ERG amplitude, as shown in Figure 1. First, because there was no measurable a-wave in the cone response, we measured the a-wave in the mixed response and recorded it as the rod a-wave (Fig. 1, downward arrow). Second, we measured the amplitude of the b-wave in the cone response (Fig. 1, right double-headed arrow). Third, we measured the b-wave in the mixed response, subtracted the cone response to obtain an isolated rod response, and measured the b-wave in that waveform (left double-headed arrow).
was considered to represent a statistically significant difference.

P23H-3 Retina values, (2) the 1 week versus the 2-week recovery values, and (3) the contrasts to compare (1) the control versus the 1 week photopic model with an autoregressive error structure. The retinal thickness and response. The residual sum of squares was also partitioned according to interactions with the above contrasts. Finally, the repeated measure-ments to the peak of the b-wave. Because an a-wave could not be elicited in the cone response, the a-wave has the same amplitude in the mixed and rod responses.

FIGURE 1. Representative ERG responses, from an adult P23H-3 rat. One is the response of the dark-adapted retina to a single flash (44.5 cd ∙ s ∙ m⁻²), and contains both rod and cone components (mixed response). One response is to the same flash, preceded at 395 ms by a rod-saturating flash (12 cd ∙ s ∙ m⁻²) and shows the cone component (cone response). The third trace (rod response) was obtained by subtracting the latter from the former. Down arrow: the rod a-wave was measured from the baseline to the early negative peak. Double-beaded arrows: the cone and rod b-waves were measured from the negative peak of the a-wave if present to the peak of the b-wave. 

Only responses recorded when body temperature was in the range 36.8 ± 57.6 °C were included. Seven animals raised in scotopic (5-lux cyclic) ambient light were studied. In each, the ERG was recorded before and after a 1-week exposure to photopic (300-lux cyclic) ambient light, and then 2 and 5 weeks after the animal was returned to scotopic conditions.

Statistical Analyses

To account for interanimal variability in ERG responses, the ERG data were analyzed as a randomized complete-block design, with the seven animals used as blocks and the interaction between animals and the four treatments as residual. The analysis of variance was then submod-eled to investigate the contrasts between (1) the control and the other treatments, (2) the 1-week response versus the 2-week recovery response, and (3) the 1-week response versus the 5-week recovery response. The residual sum of squares was also partitioned according to interactions with the above contrasts. Finally, the repeated measure-ments on the animals were investigated by using a generalized linear model with an autoregressive error structure. The retinal thickness and cone OS length data were analyzed by an analysis of variance using contrasts to compare (1) the control versus the 1 week photopic values, (2) the 1 week versus the 2-week recovery values, and (3) the 1-week versus the 5-week recovery values. For all analyses, P < 0.05 was considered to represent a statistically significant difference.

RESULTS

Effects of Ambient Light on the Function of the P23H-3 Retina

Ambient Light-Induced Loss and Recovery of the Cone and Rod b-Waves. The amplitude of the cone b-wave fell after a 1-week exposure to 300-lux cyclic light (Figs. 2A, 2B), then recovered significantly over the next 2 to 5 weeks (Figs. 2C, 2D). As shown in Figure 3A for seven different animals, each recorded serially, the amplitude of the cone b-wave varied considerably between animals. In every animal, however, 1-week exposure to photopic ambient light reduced the b-wave by approximately 50%, and reduction of illumination to scotopic levels for 2 to 5 weeks led to a recovery of amplitude. When values were averaged over the seven animals studied at each time point (Fig. 4A), the fall and recovery were both statistically significant (Table 1). When the data for each animal were normalized to the control value, photopic ambient light reduced the cone b-wave, on average, to 57% of the control value. From this, it recovered to 87% of the control value after 5 weeks in scotopic conditions. By the same analysis, the amplitude of rod b-wave was reduced by a 1-week exposure to photopic conditions to 56% of its control value, and recovered in scotopic conditions (Figs. 3B, 4B) to 78% of control, in a pattern very similar to the cone b-wave. The fall and recovery in amplitude of the rod b-wave were statistically significant (Table 1).

Ambient Light-Induced Loss and Recovery of the Rod a-Wave. The amplitude of the rod a-wave fell sharply after a 1-week exposure to 300-lux cyclic light (Figs. 2E, 2F), then recovered significantly over the next 2 to 5 weeks (Figs. 2G, 2H). When quantified (Figs. 3C, 4C), it was evident that the amplitude of the rod a-wave was reduced by the 1-week photopic ambient light to 45% of control value and then recovered to 63% of control. The reduction of the a-wave at the end of a 1-week exposure to photopic ambient light and the recovery after 2 and 5 weeks in scotopic conditions were significant (Table 1). The reduction and recovery of the a-wave confirm earlier reports from this laboratory.19,21

Effects of Ambient Light on Morphology of the P23H-3 Retina

Relative Stability of the ONL. The marked reductions of cone and rod responses caused by the 1-week exposure to photopic ambient light were associated with a limited thinning of the ONL (Fig. 5; Table 2), confirming Jozwick et al.19,21 The slow thinning of the ONL continued during the recovery of cone and rod function, over the 5-week period examined. In the present data the reduction in thickness reached statistical significance in the vulnerable superior region of retina after a 2- and 5-week recovery. Thinning of the inferior retina was sig-nificant only at the 5-week recovery time point. In a minority of animals, the ONL thinned appreciably during the 2- to 5-week recovery period, over a small area of superior midperipheral retina. In these animals, the recovery of the ERG occurred despite this localized area of photoreceptor death.

Lability of Cone and Rod Outer Segments. Both cone and rod OSs, identified by opsin immunohistochemical labeling, were markedly reduced in length by a 1-week exposure to photopic ambient illumination (Figs. 6A, 6B). During the 5-week recovery in scotopic conditions, both cone and rod OSs lengthened substantially (Figs. 6C, 6D). Quantitatively, exposure to photopic light for 1 week resulted in a 61% reduction of cone OS length. After a 5-week recovery in scotopic condi-tions, cone OS length regrew to 89% of the control value (Fig. 7). Both the reduction and recovery of cone OS length were significant (Table 3). The relatively low density of cone OSs, approximately 2000/mm² in the midperiphery, meant that the distribution of individual cones could be clearly seen in L/M opsin-labeled wholemount preparations. The lability of cone OS length in response to changes in ambient light levels was clear in these preparations (Figs. 6E-1). After 1 week in photopic conditions (Figs. 6F, 6J) the OSs were still present but much shorter. After 2- and 5-week recovery in scotopic conditions (Figs. 6G, 6K, 6L), cone OSs had regained length. Their density remained close to 2000/mm² throughout.

In 0.5-μm-thick sections of blocks taken from central retina and prepared for electron microscopy, cone and rod OSs are difficult to distinguish, but their overall shortening and re-growth were confirmed (Fig. 8).
Topography of the Shortening and Regrowth of Cone Outer Segments. The effects of light exposure and restriction on OS structure were not uniform across the retina. Two nonuniformities were observed. First, in control retinas, OSs of cones were shorter and more damaged at the anterior edge of the retina than in the mid periphery (compare Fig. 6M with Figs. 6E, 6I). This confirms a previous report of chronic photoreceptor damage at the anterior edge of the retina. OSs at the edge were not further damaged by the 1-week exposure to photopic ambient light and, correspondingly, did not repair themselves in the subsequent 2 to 5 weeks in scotopic conditions (Figs. 6N–P). Second, the shortening effect of 1 week in 300 lux was consistently more severe in superior than inferior retina (compare Figs. 6F, 6J). It is suggested in the Discussion section that these nonuniformities are determined by the prior stress experience of the retina during rearing.

Recovery of Cone and Rod Ultrastructure. Of the several structural criteria by which rods and cones have been distinguished in the electron microscope (reviewed in Refs. 24–26), the OS disc ultrastructure was the most useful in this study. In the P23H-3 rat retina, cone and rod OSs were similar in diameter, and both extended to the retinal pigmented epithelium (RPE) without tapering. Cone OSs were not obviously associated, as they are in the Nile rat, with a cone matrix or specialized regions of the RPE. Both rods and cones lacked regular well-defined incisures. However, at the level of the disc membranes, the distinction was possible. Rod OSs could be identified by membranous discs forming internal cisternae enclosed by a separate plasma membrane (Fig. 9A), while discs of cone OSs were continuous with the plasma membrane for much of their length, so that the cone OS edge appeared corrugated, without a separate border of unfolded plasma membrane (Fig. 10A). This morphologic distinction is not absolute, as discussed previously.24–26 Over short stretches, the plasma membrane in rods may be lost, and the outer membranes of several adjacent cone discs may fuse. Positive iden-

![Sample intensity series](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933445/)

**Figure 2.** Sample intensity series. In each case the retina was dark adapted, and the responses were elicited to flashes with intensities from \(4.45 \times 10^{-7}\) to \(4.45 \times 10^7\) cd \(\cdot\) s \(\cdot\) m\(^{-2}\). All traces are from the same animal, recorded serially. (A–D) These responses were recorded to a flash given 395 ms after a conditioning flash. The conditioning flash saturates the rods at the time of the second flash, so that the responses obtained represent cone activity. (E–H) These responses were recorded without a preconditioning flash and include contributions from both rods and cones. The amplitudes of both cone and rod responses were reduced by 1 week of exposure to photopic light and then recovered substantially after 2 to 5 weeks' recovery in scotopic conditions.
tification of cones was therefore restricted to instances in which the appearance of the OS border was consistent, coupled where possible with an undulation or rippling of disc membranes, described as characteristic of cones, and with particularly conspicuous concentrations of mitochondria in the ellipsoid region of the inner segment. Cones were less clearly defined in control P23H-3 retinas (Figs. 10B, 10C) than in the SD retina (Figs. 10A, 10G).

After a 1-week exposure to 300 lux ambient light, the array of OSs was disorganized. Rods and cones could not be distinguished, as their internal disc structure was severely distorted. Many OSs had extensive regions of swollen, delaminated, and vesiculated discs (Fig. 9B). Other OSs appeared condensed, and some could be identified as OSs only by their attachment to the cilium (Fig. 9C). We noted areas in which numerous disconnected lengths of OSs several micrometers long abutted the apical surface of the RPE, suggesting that the quantity of discarded membrane was such that the RPE had incorporated it only incompletely. There was no clear evidence in the 1-week exposure material that cone OSs remained less affected than rod OSs. Considering the degree of disruption of rod OSs, cone OSs should have been easily detectable, had they remained undamaged.

After 2 weeks’ recovery, and even more after 5-weeks’ recovery (Fig. 8C), well-organized OSs were again apparent, and their fine structure was generally indistinguishable from control scotopic-reared retinas (compare Figs. 9A, 9D). Cone OSs (Figs. 10D–F) were again recognizable. Many OSs were elongated and highly parallel in arrangement. At both recovery times, we detected patches of retina in which there was no regrowth of OSs. The ONL was thin, and the RPE was close to the outer limiting membrane. In most of the retina, however, there was evidence of significant regrowth of OSs.
**DISCUSSION**

**Rapid Damage of Cones by Ambient Light in a Rod-Specific Mutant Strain**

The present results show that the visual responsiveness of cones in the P23H-3 retina is rapidly and significantly reduced by a modest increase in ambient illumination, in parallel with a reduction in rod function. Within a week of exposure to photopic (300 lux) cyclic ambient conditions, both cone and rod responses were reduced to approximately half their amplitude. Morphologically, this reduction cannot be explained by photoreceptor loss, as the ONL typically remained almost constant in thickness and the density of L/M cones remained constant but can be explained by a marked shortening of OSs (Figs. 3, 4, 5). This loss occurred much more rapidly than the normal age-correlated loss of cone function in scotopic rearing, which is delayed significantly after the loss of rods.31 We are currently comparing the rates of cone and rod deterioration during the 1-week exposure to photopic conditions, to see whether a temporal sequence can be established.

**Induction of Cone Recovery by Light Restriction**

When, after the 1-week exposure to photopic conditions, ambient light was returned to scotopic levels (light restriction), OSs of cones and rods lengthened, and the corresponding ERG b-wave was returned to scotopic levels (light restriction), to see whether a temporal sequence can be established.

**FIGURE 5.** During the 6-week course of the experiment, the ONL thinned slightly, presumably reflecting the underlying death of photoreceptors in the P23H-3 strain. Thinning of the ONL was most pronounced in the superior retina. The histograms show means and standard errors (n = 5); *significantly different from the control (Table 2).

**Table 1. Reduction and Recovery of the ERG in the P23H-3 Rat in Response to Variations in Ambient Light**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>ERG Amplitude (µV)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cone b-wave</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs. 1-wk photopic</td>
<td>7 vs. 7</td>
<td>351 ± 32 vs. 201 ± 23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1-wk photopic vs. 2-wk recovery</td>
<td>7 vs. 7</td>
<td>201 ± 23 vs. 268 ± 24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1-wk photopic vs. 5-wk recovery</td>
<td>7 vs. 7</td>
<td>201 ± 23 vs. 303 ± 22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rod b-wave</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs. 1-wk photopic</td>
<td>7 vs. 7</td>
<td>529 ± 52 vs. 287 ± 34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1-wk photopic vs. 2-wk recovery</td>
<td>7 vs. 7</td>
<td>287 ± 54 vs. 357 ± 25</td>
<td>0.028</td>
</tr>
<tr>
<td>1-wk photopic vs. 5-wk recovery</td>
<td>7 vs. 7</td>
<td>287 ± 54 vs. 390 ± 30</td>
<td>0.017</td>
</tr>
</tbody>
</table>

* Non-normalized; mean ± SEM.
† By analysis of variance.

**Table 2. Changes in Retinal Thickness in the P23H-3 Rat Retina in Response to Variations in Ambient Light**

<table>
<thead>
<tr>
<th></th>
<th>ONL: OLM–ILM Distance†</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior retina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs. 1-wk photopic</td>
<td>0.27 ± 0.01 vs. 0.27 ± 0.01</td>
<td>0.789</td>
</tr>
<tr>
<td>1-wk photopic vs. 2-wk recovery</td>
<td>0.27 ± 0.01 vs. 0.22 ± 0.01</td>
<td>0.032</td>
</tr>
<tr>
<td>1-wk photopic vs. 5-wk recovery</td>
<td>0.27 ± 0.01 vs. 0.19 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inferior retina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs. 1-wk photopic</td>
<td>0.28 ± 0.02 vs. 0.27 ± 0.02</td>
<td>0.705</td>
</tr>
<tr>
<td>1-wk photopic vs. 2-wk recovery</td>
<td>0.27 ± 0.02 vs. 0.26 ± 0.02</td>
<td>0.751</td>
</tr>
<tr>
<td>1-wk photopic vs. 5-wk recovery</td>
<td>0.27 ± 0.02 vs. 0.20 ± 0.02</td>
<td>0.030</td>
</tr>
</tbody>
</table>

n = 5 in each group.
* Mean ± SEM.
† By analysis of variance.
function and metabolism, causing an increase in oxygen tension in outer retina,6 and that this rise causes oxidative stress to both rods and cones.10 This is the oxygen toxicity hypothesis, previously proposed.4

Nonuniform Effect of Light on Cones across the Retina

The insensitivity of photoreceptors at the anterior edge of the retina to ambient light conditions (Fig. 6) probably results from the chronically stressed status of the retinal edge. Photoreceptors and Müller cells at the retinal edge express high levels of stress-inducible factors (FGF-2, CNTF, and GFAP).23 Rod and cone OSs are shortened and distorted and opsin accumulates to abnormally high levels in their somas. In long-lived retinas such as that of humans, the retinal edge is eroded by a slow but progressive cystoid degeneration34,35 with pigmentary infiltra-

![Figure 6](https://iovs.arvojournals.org/figure/6.jpg)

**Figure 6.** Immunohistochemical labeling of photoreceptor OSs using antibodies for cone L/M opsin (green) and rhodopsin (red). (A–D) In retinal sections, the OSs of cones and rods was markedly reduced in length by a 1-week exposure to photopic ambient illumination and regrew significantly over the recovery period. (E–P) The shortening and regrowth of cone OSs was also seen in wholemount preparations, where shortening was more severe in superior (E–H) than in inferior (I–L) midperipheral regions of retina. Cone OSs at the anterior edge (M–P) of the retina were shorter than in the midperiphery and did not shorten further in response to photopic light exposure. Scale bars, 50 μm.

![Figure 7](https://iovs.arvojournals.org/figure/7.png)

**Figure 7.** Quantitative analysis of immunolabeled L/M opsin cone OSs. Exposure to photopic ambient light for 1 week substantially reduced the length of cone OSs. After a 2- to 5-week recovery in scotopic conditions, cone OSs regrew significantly, and their length approached that of the control. The histograms show means and standard errors (n = 5); *significantly longer than the 1-week, 300-lux OSs (Table 1).

### Table 3. Changes in L/M Cone OS Length in the P23H-3 Rat Retina in Response to Variations of Ambient Light

<table>
<thead>
<tr>
<th></th>
<th>OS Length (μm)*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs. 1-wk photopic 1-wk photopic vs. 2-wk recovery</td>
<td>16.7 ± 0.54 vs. 6.53 ± 0.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1-wk photopic vs. 5-wk recovery</td>
<td>6.53 ± 0.43 vs. 14.97 ± 0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>6.53 ± 0.43 vs. 14.25 ± 0.22</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

n = 5 in each group
* Mean ± SEM.
† By analysis of variance.
tion of the residual retina, comparable to the pigmentation in retinitis pigmentosa. We have argued that these changes are induced by chronic hyperoxic stress beginning early in life. Because their OSs are short and damaged and their expression of the metabolic enzyme cytochrome oxidase is downregulated and because factors such as FGF-2 are known to suppress responsiveness to light, the photoreceptors at the edge of the retina may be only poorly functional. Because they are conditioned to stress, however, they are relatively resistant to light damage and to increased ambient light (above). In the terms of previous studies, the edge of the retina is preconditioned by hyperoxic stress, making it resistant to the stress caused by increased ambient light.

The superior-inferior difference in the vulnerability of photoreceptors to photopic ambient light, confirming Semple-Rowland and Dawson, may also be determined by preconditioning of the retina. The difference adds to previous evidence that, in rodents, the superior retina is more vulnerable to light damage. The reason for this difference probably lies in the location of the source of ambient light in the ceiling of the holding rooms used. By the time the exposure to photopic light began, the inferior retina had had more exposure to light and, even though the light was dim (5 lux), was preconditioned.

**Clinical Relevance**

The vulnerability of cones to the degeneration of rods became clear clinically when rod–cone dystrophies, in which loss of cone vision follows loss of rod vision, were shown to result from mutations in genes expressed specifically in rods (especially rhodopsin). Loss of cone structure and function follows the loss of rod function, both spatially and temporally, even though cones do not express the product of the mutant gene. The present study shows the same vulnerability in an animal model, and demonstrates that cones recover function as rods recover function. The evidence of recovery of cone function with light restriction reinforces recent calls for the trial of light restriction as therapy in selected human cases.

Several studies have shown the OSs of rods in the nondegenerative albino SD strain shorten and show signs of membrane damage when ambient light is raised and regrow the OSs with less damaged membranes when ambient light was restricted. This shortening and lengthening of rods in response to ambient light gave rise to the concept of photostasis, which describes the response of rods in the rat retina to variations in ambient light. The responses of rods and cones in the P23H-3 retina to variations in ambient light can be viewed as an exaggerated form of photostasis, in which both the shortening of and the damage to OSs are more marked than in the wild-type retina. The clinically important point is that the capacity of photoreceptors to regrow and repair their OSs when ambient light is reduced is present in the wild-type retina.

![Figure 8](image1.jpg)

**Figure 8.** Representative light micrographs of epoxy sections of P23H-3 retinas showing regrowth of OSs. (A) Retina from a control animal raised under scotopic illumination. (B) After a 1-week exposure to photopic light, photoreceptor OSs (between arrows) appeared shortened and disorganized. (C) After a 5-week recovery in scotopic conditions, the length and regularity of OSs approached those of controls. Scale bar, 10 μm.

![Figure 9](image2.jpg)

**Figure 9.** Electron micrographs of P23H-3 rod OSs. (A) Ciliary connection from inner segment to OS. Note the wavy distortion of the nascent disc membranes at the base of the OS. The mature disc structure is apparent at right in a neighboring rod, showing the plasma membrane external to the edges of the discs. (B, C) Exposure to 300-lux cyclic ambient light for 1 week had a devastating effect on the organization of most OSs. All were shortened, and in some (B), disc stacking was severely distorted and vesiculated, whereas in others (C), the OS was recognizable only by its attachment to a ciliary process (arrow). (D) After a 5-week recovery in 5-lux ambient conditions, almost all OSs present were well organized. Ciliary region, section plane orthogonal to that of (A). Scale bars, 0.5 μm.
photoceptors, and should be available in many forms of mutation-induced photoreceptor degeneration.

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References