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 regrowth 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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One vision is affected in virtually all forms of retinal degeneration, even though many of the mutations known to cause photoreceptor degeneration affect proteins expressed specifically in rods. The loss of cone vision is therefore considered secondary to damage to rods. For the individuals affected, however, the loss of cone vision is particularly debilitating, and its amelioration is a primary concern.

Two mechanisms have been proposed that might lead from rod damage to cone damage. One is that rods produce a factor essential for cone survival1–3 and that rod loss reduces the expression of this factor below the levels required for cone integrity. In 1999, we4 suggested a more general mechanism, involving oxidative damage. We proposed that depletion of the photoreceptor population (rod or cone) by any cause would reduce consumption of oxygen flowing from the choroidal circulation. Because this flow of oxygen is unregulated, photoreceptor depletion will cause a chronic increase in oxygen tension in the outer retina.5,6 and this increase in oxygen tension is toxic to surviving photoreceptors (cones as well as rods). The vulnerability of photoreceptors to hyperoxia has also been confirmed,7–9 and evidence has been reported that rod loss results in oxidative damage to cones.10 We further proposed that this chronic hyperoxia would cause sublethal damage to surviving photoreceptors and that the damage may be reversible, allowing the restoration of photoreceptor function.

The idea that limiting the exposure of the retina to light might slow retinal degeneration goes back over 100 years (cited in Ref. 12). In animal models, including the Royal College of Surgeons (RCS) rat,13,14 the P23H-3 transgenic mouse,15,16 and rat,17 and the rhodopsin-mutant dog,18 light restriction (typically dark-rearing) has been shown to slow, or exposure to light to accelerate, retinal degeneration. In humans who have retinal degeneration, however, attempts to slow the degeneration by light restriction have reported mixed success. Berson19 reported no effect, while Stone et al.1 reported slowing of visual field loss in 10 of 14 patients. Several recent reports have called for the trial of light restriction in human retinal degenerations, based on evidence that retinas undergoing many forms of degeneration are abnormally sensitive to damage by light10,19 and that restriction slows mutation-induced degenerations (reviewed in Ref. 20) and can reverse damage to rod structure and function caused by physiological variations in ambient light.21 In this study we confirm that rods in the P23H-3 retina are hypersensitive to physiological increases in ambient light,19 when compared to a similarly pigmented control Sprague-Dawley strain, and can recover structure and function when ambient light levels are reduced.21 We test whether cones are damaged by the same light exposure and can recover structure and function when light levels are reduced. The results give insight into the dependence of cones on rod function and demonstrate the capacity of cones to recover structure and resume function after damage.

METHODS

Animals

All procedures were in accord with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the requirements...
of The Australian National University Animal Experimentation Ethics Committee. Observations were made in transgenic rats containing the P23H mutation on the rhodopsin gene (Line 3, from Beckman Laboratories, University of California, San Francisco). P23H-3 homozygous animals were established as a breeding colony. The animals used in the present experiments were heterozygotes, the offspring of mating P23H-3 homozygotes with Sprague-Dawley (SD) albinos.

**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) for 1 week (1w) and then returned to scotopic conditions for up to 5 weeks (5w). The status of photoreceptors was assessed (as below) before the exposure to photopic conditions, at the end of the 1 week exposure, and 2 and 5 weeks after exposure. The electroretinogram (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 euthanized 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 3 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 (1:110, Rhod2D2, 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 PBS 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 DNAspecific dye bisbenzamide (10,000) 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 microwave on ice using six 10-second bursts at 10-second intervals, at 80 mW in a histologic microwave oven (Peleco 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 (Ultracut E; Leica), stained with uranyl acetate alone or followed by Reynold’s 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 bisbenzamide. 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.

**Electroretinography**

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 establish consistent recording conditions, we recorded responses to a range of test flash intensities (4.45 × 10⁻² to 4.45 × 10⁻³ cd⋅s⋅m⁻²). For the quantitative comparisons in Figures 3 to 4, we recorded responses to a standard test flash (4.45 × 10⁻³ cd⋅s⋅m⁻²) and then repeated the recordings with the test flash, preceded by 395 ms by a condition- ing flash (12 cd⋅s⋅m⁻²). After previous reports, the unconditioned response can be considered to be mixed, with contributions from rods and cones. The conditioned responses are those of cones and, by subtracting the latter from the former, the rod response can be isolated.

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.

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

RESULTS 1-week versus the 5-week recovery values. For all analyses, (2) the 1 week versus the 2-week recovery values, and (3) the contrasts to compare (1) the control versus the 1 week photopic ambient light were associated with a limited thinning of the ONL (Fig. 5; Table 2), confirming Jozwick et al.\textsuperscript{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 significant 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 light (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 conditions, 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/mm\textsuperscript{2} 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–L). 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, 6L), cone OSs had regained length. Their density remained significantly 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.

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

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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 regrowth 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,27 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-

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**FIGURE 2.** Sample intensity series. In each case the retina was dark adapted, and the responses were elicited to flashes with intensities from $4.45 \times 10^{-3}$ to $4.45 \times 10^7$ cd·s·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,28–30 and with particularly conspicuous concentrations of mitochondria in the ellipsoid region of the inner segment.26 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.

FIGURE 3. Amplitudes of the ERG cone b-wave (A), rod b-wave (B) and rod a-wave (C) for seven P23H-3 animals. The stimulus used for all measures was a flash of 44.5 cd·s·m⁻²-intensity. For each animal, four values are shown: the amplitudes recorded before (control) and immediately after the 1-week exposure to photopic ambient light and the amplitudes recorded 2 and 5 weeks after the animal was returned to scotopic conditions. The absolute values of the signal varied, but in each case exposure to photopic light exposure reduced amplitude, and the amplitude recovered in scotopic conditions.

FIGURE 4. Average amplitudes of the cone b-wave (A), rod b-wave (B), and rod a-wave (C). The stimulus used for all measures was a flash of 44.5-cd·s·m⁻² intensity, as in Figure 3. The histograms show means and standard errors for the seven animals studied. The asterisks on the 2- and 5-week recovery values indicate that they are significantly larger than the 1-week, 300-lux value (Table 1).
**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. 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 components recovered amplitude. The magnitude of the damage and recovery was similar in rods and cones (Fig. 4), at the time points examined. More detailed testing will be needed to test whether the damage and recovery of rods can be shown to precede that of cones. Cone function (the cone b-wave) recovered over 5 weeks to 85% of control values, and we are currently testing the level of recovery achieved after longer periods of reduced ambient light. This is the first description of cone recovery induced by light restriction.

**Link between Rod Damage and Cone Damage**

Because the P23H-3 transgene is a rhodopsin mutation, it is a reasonable initial assumption that the breakdown of photoreceptor structure and function induced by photopic ambient light begins with rods. Correspondingly, in scotopic rearing conditions, the cone component of the ERG maintains amplitude for many weeks after the rod component declines. In the present experiments, by contrast, both cone and rod function declined rapidly (within 1 week), and it seems possible that rods and cones are damaged by the same stress. We have suggested previously that the mechanism of light-induced damage is oxidative stress. In a comparable model of cone damage after the degeneration of rods, evidence of oxidative stress to the surviving cones has been identified, and antioxidants have been shown to slow both rod and cone death in rodent models of photoreceptor degeneration. A viable hypothesis therefore is that light induces a reduction of rod

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Condition 1</th>
<th>Condition 2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cone b-wave</td>
<td>Control vs. 1-wk photopic</td>
<td>7 vs. 7</td>
<td>351 ± 32 vs. 201 ± 23</td>
</tr>
<tr>
<td></td>
<td>1-wk photopic vs. 2-wk recovery</td>
<td>7 vs. 7</td>
<td>201 ± 23 vs. 268 ± 24</td>
</tr>
<tr>
<td></td>
<td>1-wk photopic vs. 5-wk recovery</td>
<td>7 vs. 7</td>
<td>201 ± 23 vs. 303 ± 22</td>
</tr>
<tr>
<td>Rod a-wave</td>
<td>Control vs. 1-wk photopic</td>
<td>7 vs. 7</td>
<td>529 ± 52 vs. 287 ± 34</td>
</tr>
<tr>
<td></td>
<td>1-wk photopic vs. 2-wk recovery</td>
<td>7 vs. 7</td>
<td>287 ± 34 vs. 357 ± 52</td>
</tr>
<tr>
<td></td>
<td>1-wk photopic vs. 5-wk recovery</td>
<td>7 vs. 7</td>
<td>287 ± 34 vs. 390 ± 30</td>
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</table>

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

**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).
function and metabolism, causing an increase in oxygen tension in outer retina, and that this rise causes oxidative stress to both rods and cones. This is the oxygen toxicity hypothesis, previously proposed.

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). 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 degeneration with pigmentary infiltration.

**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>OS Length (μm)</th>
<th>Control vs. 1-wk photopic</th>
<th>1-wk photopic vs. 2-wk recovery</th>
<th>1-wk photopic vs. 5-wk recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5 lux)</td>
<td>16.7 ± 0.54 vs. 6.53 ± 0.43</td>
<td>6.53 ± 0.43 vs. 14.97 ± 0.27</td>
<td>6.53 ± 0.43 vs. 14.25 ± 0.22</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.25 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,36 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,58-59 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,40 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.57 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).1 Loss of cone structure and function follows the loss of rod function, both spatially and temporally,1,41 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.4,20

Several studies21,40,42 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,43,44 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

![Figure 8](image-url) 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 short 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](image-url) 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.

Acknowledgments

The authors thank Emlyn R. Williams (Statistical Consulting Unit, The Australian National University) for advice and help with the analysis of data.

References

21. Jozwick C, Valter K, Stone J. Reversal of functional loss in the P23H-3 rat after 2-week recovery. Identiﬁcation is conﬁrmed by the concentration of mitochondria (arrow) in the inner segment (compare rods in Fig. 9). (E) Cone border in a P23H-3 rat after a 5-week recovery. Scales: (A, B, D, F) 500 nm; (C, E, G) 200 nm.

FIGURE 10. Electron micrographs of cone Oss. Cones (labeled Co in A, B, D) are identiﬁed by the lack of an outer membrane surrounding the disc stack, and by varying degrees of “waviness” of the disc membranes when compared to rod Oss (B). The regions clearly lacking a surrounding membrane are shown in detail in (C), (E), and (G). (A, G) Young adult SD rat raised in 5-lux illumination. (B, C) Control P23H-3 rat raised in 5-lux illumination. (D, E) Cone from P23H-3 rat after a 2-week recovery. Identifcation is conﬁrmed by the concentration of mitochondria (arrow) in the inner segment (compare rods in Fig. 9). (E) Cone border in a P23H-3 rat after a 5-week recovery. Scales: (A, B, D, F) 500 nm; (C, E, G) 200 nm.


