Increased Susceptibility to Constant Light in *nr* and *pcd* Mice with Inherited Retinal Degenerations

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**Purpose.** To determine whether the degenerating photoreceptors in nervous (*nr/nr*) and Purkinje cell degeneration (*pcd/pcd*) mutant mice are more susceptible to the damaging effects of constant light than those in age-matched normal mice.

**Methods.** Beginning at two ages for each mutant, albino *nr/nr* and *pcd/pcd* mice were placed into constant fluorescent light at an illuminance of 115 foot-candles to 130 foot-candles for a period of 1 week. Age-matched (usually littermate) normal (+/−) mice were exposed at the same time. The degree of photoreceptor cell loss was quantified histologically by obtaining a mean outer nuclear layer thickness for each animal. The light-exposed mice were compared with age-matched mutant and normal mice that were maintained in cyclic light.

**Results.** The homozygous mutants at each age showed a significantly greater loss of photoreceptor cells caused by constant light exposure than did the normal +/− mice in the same period of light exposure. The *nr/nr* and *pcd/pcd* mutants lost two to three times the number of photoreceptor cells.
The interaction of environmental light and inherited and age-related retinal degenerations has been the subject of considerable interest since the finding of Dowling and Sidman\(^1\) that dark-rearing of rats with inherited retinal dystrophy can slow the pace of photoreceptor degeneration. Indeed, this observation led, in part, to the testing of light deprivation as an experimental therapeutic measure for patients with early stages of retinitis pigmentosa.\(^2\)

On the negative side, it has been demonstrated repeatedly that constant or excessive light is harmful to photoreceptor cells, even in otherwise normal retinas. Without significant experimental evidence, excessive light has long been held to be harmful to patients with ongoing retinal degenerations,\(^3\) and many ophthalmologists have recommended dense sunglasses for their patients. Excessive light has also been implicated in the etiology of age-related macular degeneration.\(^4\)

There have been a few experimental reports supporting the notion that photoreceptor cells undergoing an inherited retinal degeneration are more susceptible to the damaging effects of light than are normal photoreceptors. Noell\(^5\) described a dramatic sensitivity to light of rats with inherited retinal dystrophy, suggesting that a 1-day exposure advances the "functional age" of the retinal degeneration by approximately 30 days. Likewise, albino homozygous retinal degeneration slow \((rds/rds)\) mice, and \(rds+/+\) heterozygotes with even slower degeneration, have been found to be more vulnerable to constant light than age-matched normal mice.\(^6\) Similar findings have more recently been obtained with transgenic mice with a mutant opsin gene.\(^7\) To determine whether retinal degenerations generally predispone photoreceptors to a greater susceptibility to environmental light, and because of the potential clinical importance of this topic, we have asked whether excessive light has a particularly deleterious effect in those two additional murine retinal degenerations, those found in the nervous \((nr/nr)\) and Purkinje cell degeneration \((pcd/pcd)\) mutant mice.

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### Materials and Methods

The \(nr/nr\) and \(pcd/pcd\) mice were born in our laboratory and were maintained in cyclic fluorescent light at an illuminance level of less than 15 foot-candles (fc-c). The \(nr/nr\) mice were coisogenic with the albino BALB/c strain,\(^8\) and the albino \(pcd/pcd\) mice were derived from outcrosses of mice of the pigmented C57BL/6j \(pcd\) background with albino BALB/c mice as described previously.\(^9\) Because most of our breeding crosses were heterozygote \(x\) heterozygote, homozygous mutants comprised only approximately 25% of each litter. These homozygotes could be distinguished unequivocally from their normal littermates by the presence of cerebellar ataxia by 30 to 35 days of age.\(^9\) The normal mice were either heterozygotes or homozygous wild-type, which could not be distinguished so their genotypes were thus designated \(+/-\). At two ages for each of the mutants (Fig. 2), when the number of photoreceptor nuclei in the outer nuclear layer (ONL) was reduced to approximately 70% to 80% of normal and later, when it was reduced to 40% to 50% of normal, the animals were placed into constant fluorescent light at an illumination level of 115 fc-c to 130 fc-c for 1 week, as described elsewhere.\(^10\) Normal \(+/-\) mice were also exposed to constant light, and homozygous mutants and normal \(+/-\) mice were kept for the same time in cyclic light to serve as controls. In most cases, littersmates were used in the experiments, and those receiving constant light were exposed in the same cage.

After light exposure, the mice were then killed by overdose of carbon dioxide followed immediately by enucleation and immersion-fixation of their eyes with mixed aldehydes. All animal procedures adhered to the ARVO Resolution on the Use of Animals and the guidelines of the University of California San Francisco Committee on Animal Research. The eyes were embedded in epoxy resin and sectioned at 1 \(\mu\)m thickness along the vertical meridian.\(^10\) The thickness of the ONL was taken as a measure of photoreceptor cell number\(^10\) and was obtained by taking 54 measurements (27 each in the superior and inferior hemispheres) at defined points around the eye with the aid of a Bioquant morphometry system (R and M Biometrics, Nashville, TN) as described elsewhere.\(^10\) A mean ONL thickness was obtained for each mouse from these 54 measurements.

The percent reduction of ONL thickness due to constant light damage was calculated from the mean values of the cyclic light-maintained and the light-exposed mice for each age and genotype. In this way, the percent loss of photoreceptor cells could be compared between groups (Table 1). For statistical analysis, the percent reduction for each individual light-ex-
posed mouse was determined by comparing its ONL thickness to that of the average value of all the cyclic light-maintained mice of the same age and genotype. Statistical comparisons of the percent reductions between +/- and homozygous mutant mice were then made using a two-tailed unpaired Student's t-test.

RESULTS

The results of the \textit{nr/nr} and control mice at the younger, 2-month, age will be described in some detail, and the other three groups (older \textit{nr/nr} and both \textit{pcd/pcd} ages) will be considered mostly quantitatively.

When the control +/- mice were exposed to constant light for 1 week, the photoreceptors were significantly damaged. They had lost virtually all their outer segments, and their inner segments were somewhat disrupted and shortened compared with their cyclic light controls (Figs. 1A, 1B). The ONL was reduced in thickness only approximately 16\% from that in cyclic light controls (Fig. 2A; Table 1).

The \textit{nr/nr} mice maintained in cyclic light (Fig. 1C) had the histologic appearance expected of 2-month-old animals, including the presence of significantly shortened photoreceptor inner segments and reduced volume of outer segment membranes, most of which were highly disorganized and arranged, in some cases, in whorls (Fig. 1C). The week of constant light severely damaged the \textit{nr/nr} photoreceptors (Fig. 1D), reducing the ONL in the most posterior retina to a single but complete row of nuclei, which grades up to 3 rows of nuclei in the periphery. Only very small nubs of inner segments were present in the eye, mostly in the far periphery, and almost no outer segment membranes were present anywhere in the retina (Fig. 1D). Overall, the mean ONL thickness was approximately 10\,\mu m to 12\,\mu m (Fig. 2A), which represented a reduction of just more than 50\% of the ONL thickness compared to control mice of the same genotype and age when kept in cyclic light (Table 1).

The results of the experiments with \textit{nr/nr} mice at 5 months of age were similar to those at 2 months. The normal +/- mice showed about the same degree of damage (Fig. 2A), with a loss of just more than 20\% of the ONL thickness (Table 1). The ONL in \textit{nr/nr} mice in cyclic light was significantly thinner at 5 months than at 2 months (Fig. 2A), as expected, but the relative damage to the \textit{nr/nr} homozygous mutants by constant light was proportionately about the same as at 2 months, showing about a 50\% loss of nuclei (Table 1). The differences in the degree of light-induced reduction in ONL thickness between the homozygous mutant \textit{nr/nr} mice and +/- mice (Fig. 2B) were highly significant at both ages (Table 1).

The results with the \textit{pcd/pcd} mice at both ages (Fig. 2B) were similar to those with the \textit{nr/nr} mice (Fig. 2A), except...
that the damage done by the constant light exposure appeared even greater in the pcd/pcd mice when compared with nr/nr (Fig. 2). The reduction in ONL thickness in the pcd/pcd mice from that of the age- and genotype-matched cyclic light control animals was approximately 65% to 70% at both of the ages (Table 1). The differences between the homozygous mutant pcd/pcd mice and +/- mice in the degree of light-induced reduction in ONL thickness (Fig. 2B) were highly significant at both ages (Table 1).

The differences in the degree of light damage between pcd/pcd and nr/nr mice, based on a comparison of the percent reduction of ONL thickness of the individual mice, were not statistically significant at the younger ages but were at the older ages ($P < 0.01$). (It should be noted that although the values for light-damaged +/- mice in the nr/nr line appear to be somewhat lower than those of +/- mice in the pcd/pcd line, they are not statistically different.) In both nr/nr and pcd/pcd mice, the light damage did not appear to include any changes in the retinal pigment epithelium or inner retina.

Differences in degeneration between the superior and inferior hemispheres were examined because albino rodents typically show a greater degree of light-induced degeneration in the superior than in the inferior hemisphere of the eye. After 1 week of constant light exposure, the superior hemisphere of +/- mice was, indeed, more degenerated than the inferior hemisphere. However, the difference between the two hemispheres was less than a single row of nuclei in the +/- mice exposed at 1.5 or 2 months of age and the +/- mice from the nr/nr line at 5.5 months of age, and only 1 to 2 rows different in the pcd/pcd line at 3.5 months. In the homozygous mutants, which showed significantly more degeneration, almost no difference was seen between the two hemispheres. Two factors prevent us from drawing firm conclusions about these findings. First, normal mice (unlike rats) are relatively resistant to 1-week exposures to constant light, and superior-inferior hemispheric differences are not particularly obvious unless longer exposures are used. Second, the degeneration in the homozygous mutant mice was so severe that only one to two rows of nuclei were present in the posterior retina, thereby making subtle differences very difficult to assess with confidence.

DISCUSSION

From the present studies, it is clear that nr/nr and pcd/pcd homozygous mutant mice are much more susceptible to the damaging effects of constant light than are normal mice of the same age. In each of the four groups (two ages of exposure with each mutant), constant light exposure resulted in the loss of two to three times the number of photoreceptor cells in the mutants than the loss in the age-matched normal +/- mice (Table 1). With each mutant, approximately the same degree of photoreceptor cell loss occurred at each of the two ages (Table 1).

The somewhat greater damaging effect of light exposure on the retinas of pcd/pcd mice than of nr/nr mice indicates that the insult of environmental light may interact differently with photoreceptors undergoing different cytopathologic processes. Clearly, the cytopathology of photoreceptor degeneration in nr/nr and pcd/pcd mice is different. It may be relevant that the pcd/pcd retinas showed distinct rod outer segments that became progressively shorter with age, as shown previously, whereas rod outer segment membranes in the nr/nr retinas were arranged mostly in disorganized arrays and whorls. It may be that the pcd/pcd retina contains more rhodopsin than the nr/nr retina at a comparable stage of degeneration, but this remains to be shown. If so, however, this could explain the greater degree of damage to the pcd/pcd retina, because it is thought that a greater quantity of rhodopsin in an eye leads to a greater severity of photoreceptor degeneration.

The direct relationship of the present findings to human patients with inherited and age-related retinal degenerations cannot be assessed precisely. For one reason, direct human
counterparts to the \textit{nr/nr} and \textit{pcd/pcd} mutations have not yet been identified. Moreover, the mice used in the present study were albinos, which are significantly more susceptible to light damage than pigmented eyes, such as the human eye. Indeed, in the study in which retinal \textit{rds/rds} homozygotes and \textit{rds/+} heterozygotes showed more degeneration than normal control animals, only the albinos demonstrated accelerated degeneration, whereas the pigmented mice of the same genotypes showed none.\textsuperscript{6} Nevertheless, pigmented retinal dystrophic rats\textsuperscript{5} and pigmented mutant rhodopsin transgenic mice\textsuperscript{7} did show an increased susceptibility to excessive light. Thus, eye pigmentation alone does not necessarily protect all retinas from excessive light. At the very least, our study brings to six the number of inherited retinal degenerations that have a greater susceptibility to light damage than that seen in normal control animals. These include inherited retinal degenerations that have a wide range of different mutations and, presumably, cytopathologic processes, suggests that photoreceptors undergoing many different forms of degeneration may be at greater risk than normal photoreceptors to the damaging effects of a second insult such as excessive environmental light. For this reason, it would seem prudent to limit or eliminate retinal exposure to intense light or to extended periods of bright light in patients with inherited and age-related retinal degenerations.

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\textbf{References}


