Age-related and light-associated retinal changes in Fischer rats

Yin-Lok Lai, Robert O. Jacoby, and Albert M. Jonas

Morphological changes in retinas of aging Fischer 344 rats were characterized. The numbers of photoreceptor cells gradually decreased as rats aged. The outer nuclear layer was 12 cells thick at 3 months, but was reduced to less than 8 cells by 18 months. The decrease of photoreceptor cells was more pronounced in rats housed under a light intensity of 32 ft-c than in rats housed under a light intensity of 1 ft-c. Inner and outer segments of surviving photoreceptor cells were morphologically normal. A new form of retinal degeneration was discovered in aged Fischer rats characterized by selective degeneration of peripheral retina. Degeneration was characterized by severe loss of photoreceptor cells in the far peripheral retina. Microcystoids were found in about 25% of the affected retinas, and the loss of photoreceptor cells was followed by proliferation and vascularization of the retinal pigment epithelium and disorganization of retinal structures. The incidence and severity of peripheral retinal degeneration increased with age and prolonged exposure to comparatively high-intensity light. All Fischer rats (5/5) housed under light intensity of 32 ft-c developed severe peripheral retinal degeneration by 24 months. Peripheral retinal degeneration was an age-related change but appeared to be exaggerated by ambient light.

Key words: retinal degeneration, rat retina, photoreceptor cells, retinal pigment epithelium, age-related factor, light-associated factor, peripheral retinal degeneration

Rats have been widely used for experimental eye research, but little is known about their age-related retinal changes. It was first reported that the general retinal structure and the thickness of the outer nuclear layer of the Wistar rats did not change with advanced age. However, two recent reports indicate that albino rats developed retinal degeneration after daily 12 hr exposures to less than 20 ft-c of light for more than 2 years. It was characterized by atrophy of the first neuron, partial degeneration of the second neuron, and destruction of the retinal structure as well as by vascularization of the multilayered pigment epithelium. These changes were attributed to low-intensity light damage.

This report describes age-related and light-associated retinal changes in Fischer 344 rats and discusses their pathogenesis.

Material and methods

Two groups of 1-month-old male, albino Fischer 344 rats (Charles River Breeding Laboratory, Wilmington, Mass.) were housed in opaque white plastic boxes with open-wire tops and pinechip bedding. Rats were kept in a specific pathogen-free, controlled-barrier facility, and were fed food and water ad libitum. Ambient light was provided by ceiling-mounted fluorescent lamps regulated on a 12 hr on/off cycle. Reflected light intensity from the bottom of boxes was maintained at 1 or 32 ft-c and checked at regular intervals with a Luna-Pro electronic system exposure meter.

From the Section of Comparative Medicine, Yale University School of Medicine, New Haven, Conn.
Supported by National Institutes of Health Grants RR00393 and EY01769.
Submitted for publication Nov. 30, 1977.
Reprint requests: Dr. Yin-Lok Lai, Section of Comparative Medicine, Yale University School of Medicine, 333 Cedar St., New Haven, Conn. 06510.
Fig. 1. Retinas from Fischer rats (×375). Areas shown are from midpoint between the ora serrata and optic disc. A, 3-month-old rat raised under a light intensity of 32 ft-c. The retina is well developed, and the photoreceptor layer is about 11 to 12 cells thick. B, 24-month-old rat exposed to 1 ft-c. All layers are present, but the photoreceptor layer is reduced to 7 to 8 cells. C, 24-month-old rat exposed to 32 ft-c. This retina is thinner than those in A and B, and the photoreceptor layer is about 5 to 6 cells thick. The reduced thickness of the photoreceptor layer is caused, in part, by shortening of the inner and outer segments.

Eyes from five rats exposed to each light intensity were examined at 3, 6, 9, 12, 18, and 24 months of age.

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital and were perfused through the left ventricle and aorta with 2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.4, under a constant pressure of 60 to 80 mm Hg for 10 min. Eyeballs were enucleated and dissected transversely into two parts immediately behind the limbus. The posterior eye cups were immersed in the same fixative for 2 hr at room temperature and washed briefly in 0.13M phosphate buffer. They were postfixed for 2 hr in 1% osmium tetroxide buffered with 0.1M phosphate at room temperature. Fixed eye cups were embedded in Spurr's medium. One-micron sections of the hemispheres of the eye cup, including a full length of the retina between two sides of ora serrata and the optic disc, were prepared with glass knives and stained with Azur II-methylene blue for light microscopy. Ultrastructural studies will be reported separately.

Results

The thickness of the photoreceptor layer over the entire retina in both groups of rats decreased progressively with age. For example, the photoreceptor layer in the midperiphery (midway between the ora serrata and the optic disc) was 12 cells thick in rats up to 6 months of age but gradually decreased to an average of less than 10 cells per column at 9 months and 8 cells per column at 18 months of age. Reduction in the number of photoreceptor cells was more prominent in rats housed at light intensity of 32 ft-c than in rats housed at 1 ft-c (Fig. 1). The reduction of photoreceptor cells occurred uniformly from ora serrata to the posterior pole except in two
Fig. 2. Peripheral segments of retinas of Fischer rats (×150). A, 3-month-old rat exposed to 1 ft·c. The retina is well developed, and the photoreceptor layer is about 8 cells thick. B, 24-month-old rat exposed to 1 ft·c. All retinal layers are thinner than those of young rats, and photoreceptor cells have disappeared from the far peripheral retina. The retinal epithelium and inner retinal layers remain histologically normal. A small microcystoid is shown in the inner nuclear layer at the upper part of the micrograph. C, 24-month-old rat exposed to 32 ft·c. Photoreceptor cells have almost completely disappeared from this part of the retina, and the thickness of the inner retinal layers is decreased. The retinal epithelium and inner retinal layers, however, are morphologically normal. D, 24-month-old rat exposed to 32 ft·c. Photoreceptor cells in this area of the retina have almost completely disappeared, with only few modified photoreceptor nuclei without inner and outer segments. Multiplication, migration, and vascularization of the retinal epithelium are extensive. E, 24-month-old rat exposed to 1 ft·c. A well-established retinal lesion. All photoreceptor cells have disappeared except only very few modified photoreceptor nuclei. The retinal epithelium shows profound changes, with cell multiplication, cell migration, and vascularization. Gliosis is developing in the inner nuclear layer in the lower part of this micrograph. There is a large cystoid in the center of the field and a smaller one in the lower part. Unidentified cells have proliferated along the inner surface of the retina in the uppermost part of the micrograph. F, 24-month-old rat exposed to 1 ft·c. An end-stage retinal lesion approximately 120 μ away from the ora serrata. Photoreceptor cells are completely gone, the retinal structure is unrecognizable, and cystoids have coalesced to form large cavities.

In addition to generalized thinning of the photoreceptor cell layer, a selective retinal degeneration developed at the periphery of the retina.

Peripheral retinal degeneration was graded into three stages: grade 1, a mild, early lesion in which the thickness of the photoreceptor cell layer was decreased but inner and outer segments remained intact; grade 2, a moderate lesion in which inner and outer segments were partially lost but photoreceptor nuclei were still present; and grade 3, a severe lesion in which photoreceptor nuclei were completely lost and photoreceptor cells were replaced by modified photoreceptor nuclei. In eyes from two 24-month-old rats, in which cell loss was more severe in the posterior pole of the globe. Inner and outer segments of remaining photoreceptor cells in affected retinas remained histologically normal, and retinal architecture in aged rats was not distorted.

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Table I. Morbidity and severity of peripheral retinal degeneration in eyes of aged Fischer 344 rats*

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Light (ft-c)</th>
<th>Severity</th>
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<th>Morbidity rate in examined eyes (%)</th>
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<tbody>
<tr>
<td>18</td>
<td>1</td>
<td>Grade 1 (%)</td>
<td>50</td>
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<td>50</td>
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<tr>
<td>24</td>
<td>1</td>
<td>Grade 2 (%)</td>
<td>10</td>
<td></td>
<td>90</td>
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<tr>
<td>24</td>
<td>32</td>
<td>Grade 3 (%)</td>
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<td>70</td>
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*Five rats (10 eyes) per treatment group. Results are expressed as the percentage of eyes affected.

receptor layer in the peripheral retina was reduced to about two cells or complete loss of photoreceptor cells occurred in a segment shorter than 120 μ (Fig. 2, A and B); grade 2, moderate lesion in which loss of photoreceptor cells involved segments longer than 120 μ but the pigment epithelium and the inner retinal layers remained normal (Fig. 2, C); grade 3, a late stage characterized by almost complete loss of photoreceptor cells in the affected area and proliferation and vascular invasion of the retinal pigment epithelium (Fig. 2, D).

The incidence and severity of peripheral retinal degeneration increased in rats between 18 and 24 months and were augmented in rats exposed to higher light intensity (Table I). In addition, eyes from 24-month-old rats developed mild microcystoid degeneration in the peripheral retina. The microcystoids were usually small, round, and sparse and were located in the outer portion of the inner plexiform layer (Fig. 2, B and D). In severe cases, coalescence of microcystoids into larger cystoids occurred, and cellular projections into the lumens of the cystoids were seen (Fig. 2, E). Thickening of the choroidal arteriole wall, focal increase of choroidal connective content, and vitreoretinal adhesions were observed less frequently.

Discussion

Light-induced retinal damage in rats has been well documented. Outer segments of the photoreceptor cells degenerate first, and the photoreceptor cells are involved later. Lesions are more severe in the posterior pole than in the peripheral retina. Widespread loss of photoreceptor cells in retinas of Fischer 344 rats occurred without any detectable preceding histological alterations in the photoreceptor outer segments. Remaining photoreceptors were histologically normal. Gradual loss of photoreceptor cells in this strain does not appear to be light-related, since losses occurred in retinas exposed to light of less than 1 ft-c. Therefore it is reasonable to assume that the cell loss is an age-related change. If we consider that photoreceptor cells function as specialized neurons, which are stimulated by light rays from the environment and initiate a series of physiological reactions resulting in vision, then higher intensities of environmental light may cause a heavier workload for photoreceptor cells, thus exaggerating retinal aging changes in Fischer 344 rats. This may explain why retinal degeneration is more prevalent in Fischer 344 rats housed under high light intensities. Loss of inner retinal layers was observed only after complete loss of photoreceptor cells. Focal alteration of inner retinal layers was found occasionally in younger rats but was not compatible with age-associated or light-induced damage.

Peripheral degeneration of the retina began at the far periphery of the retina, where because of the optical design of the eye, the lowest levels of light reach the photoreceptor layer. In addition, peripheral degeneration was not related to the severity of photoreceptor cell loss in other parts of the retina. Therefore peripheral retinal degeneration in Fischer 344 rats appears to be age-related rather than a result of light-induced damage.

Peripheral retinal degeneration is a common disorder of man. It includes a spectrum of peripheral retinal lesions, including cystoid degeneration, paving-stone degenera-
Peripheral retinal degeneration in aged Fischer 344 rats mimics the human disease histologically and is therefore a potentially important animal model for peripheral retinal degeneration in man.

These studies also indicate that age-related and light-associated retinal changes must be considered in long-term experiments involving rats.

REFERENCES