Development of Choroidal Neovascularization in Rats With Advanced Intense Cyclic Light–Induced Retinal Degeneration

Daniel M. Albert, MD, MS; Aneesh Neekhra, MD; Shoujian Wang, MD, PhD; Soesiaawati R. Darjatmoko, BS; Christine M. Sorenson, PhD; Richard R. Dubielzig, DVM; Nader Sheibani, PhD

Objectives: To study the progressive changes of intense cyclic light–induced retinal degeneration and to determine whether it results in choroidal neovascularization (CNV).

Methods: Albino rats were exposed to 12 hours of 3000-lux cyclic light for 1, 3, or 6 months. Fundus examination, fundus photography, fluorescein and indocyanine green angiography, and optical coherence tomography were performed prior to euthanization. Light-exposed animals were euthanized after 1, 3, or 6 months for histopathological evaluation. Retinas were examined for the presence of 4-hydroxy-2-nonenal– and nitrotyrosine-modified proteins by immunofluorescence staining.

Results: Long-term intense cyclic light exposure resulted in retinal degeneration with loss of the outer segments of photoreceptors and approximately two-thirds of the outer nuclear layer as well as development of subretinal pigment epithelium neovascularization after 1 month. Almost the entire outer nuclear layer was absent with the presence of CNV, which penetrated the Bruch membrane and extended into the outer retina after 3 months. Absence of the outer nuclear layer, multiple foci of CNV, retinal pigment epithelial fibrous metaplasia, and connective tissue bands containing blood vessels extending into the retina were observed after 6 months. All intense light–exposed animals showed an increased presence of 4-hydroxy-2-nonenal and nitrotyrosine staining. Optical coherence tomographic and angiographic studies confirmed retinal thinning and leakiness of the newly formed blood vessels.

Conclusions: Our results suggest that albino rats develop progressive stages of retinal degeneration and CNV after long-term intense cyclic light exposure, allowing the detailed study of the pathogenesis and treatment of age-related macular degeneration.

Clinical Relevance: The ability to study the progressive pathogenesis of age-related macular degeneration and CNV will provide detailed knowledge about the disease and aid in the development of target-specific therapy.


A R C H I V E S   E X P R E S S

Author Affiliations:
Departments of Ophthalmology and Visual Sciences (Drs Albert, Neekhra, Wang, and Sheibani and Ms Darjatmoko), Pediatrics (Dr Sorenson), Veterinary Medicine (Dr Dubielzig), and Pharmacology (Dr Sheibani), University of Wisconsin School of Medicine and Public Health, Madison.

©2010 American Medical Association. All rights reserved.

Downloaded From: https://jamanetwork.com/ by a Non-Human Traffic (NHT) User on 03/22/2021
nergy is essential for visual function, but in excessive amounts it produces photochemical damage to retinal neurosensory cells. In 1966, Noell et al published an early histological study of retinal damage by light in rats. They documented the loss of photoreceptors and the outer nuclear layer (ONL) in albino rats after exposing them to constant light for periods ranging from a few hours to a few days. Subsequent light and electron microscopical studies revealed the sequence of histological events leading to the disappearance of photoreceptors and complete RPE–Müller cell adhesion as well as RPE neovascularization by retinal capillaries.

In 1988, Kremers and van Norren proposed 2 classes of photochemical damage of the retina depending on the level of irradiance of exposed white light. In class 1 with low irradiance, the initial damage is restricted to photoreceptors; in class 2 with high irradiance, the RPE is the site of first insult. The rate and extent of retinal degeneration were increased with an increase in the duration and intensity of light exposure. The appearance of the albino rat retina remained normal after 1 hour of 2500-lux light exposure, but pyknotic changes in the ONL were noted when light exposure was increased to 4 hours. Additionally, O’Steen et al reported that the susceptibility to light damage in the rat increases markedly at ages 16 to 24 weeks compared with ages 3 to 4 weeks, with approximately 95% loss of photoreceptors.

The exact molecular events involved in light-induced retinal degeneration have not been fully determined. However, formation of reactive oxygen species and consequent oxidative damage to retinal cells is one of the proposed mechanisms. It is also suggested that the formation of bleached products of rhodopsin or other visual pigments leads to the formation of phototoxic molecules that mediate retinal cytotoxicity. Both the caspase-dependent and caspase-independent pathways are involved in light-induced apoptosis of photoreceptors. In recent years, vision scientists have increasingly used light-induced retinal degeneration in rodents for pathogenetic and therapeutic studies following long-term light exposure for a month or longer. However, the advanced stages of intense cyclic light–induced retinal degeneration have not been previously evaluated.

Long-term light-induced reactive oxygen species can damage the Bruch membrane and may stimulate CNV by fostering a proangiogenic environment in the retina. Numerous studies using various retinal degeneration models reported RPE neovascularization, which arises from the inner retinal capillaries with minimal contribution from the choroid vasculature and may be influenced by the rate at which photoreceptors degenerate. However, the accumulation of lipofuscin in RPE cells is, at least in part, also related to light exposure. Lipofuscin deposition contributes to the formation of large confluent drusen and pigmented changes in the RPE layer and is also thought to lead to the development of CNV. Thus, the relative contributions of the neurosensory retina and RPE degeneration to the development of CNV in the aging eye clearly require further study.

Based on the published work of others and our own previous observations of light-induced retinal degeneration in rodents, we hypothesized that rodents with late-stage intense cyclic light–induced retinal degeneration develop CNV. To test this hypothesis, we studied the effects of cyclic light exposure with long duration and high intensity on the eyes of Wistar and Sprague-Dawley (SD) albino rats. Here we show that exposure to high-intensity cyclic light for 1 to 6 months resulted in rapid retinal degeneration that ultimately progressed to CNV with minimal contribution from retinal capillaries and RPE neovascularization. These changes were associated with increased oxidative stress as revealed by increased immunostaining for the presence of 4-hydroxy-2-nonenal (HNE)–modified proteins, a product of lipid peroxidation, and nitrotyrosine-modified proteins, a product of peroxynitrate reaction. Thus, long-term intense cyclic light exposure of albino animals may provide a suitable model to study the progressive pathogenesis of exudative AMD and be useful in the development of targeted treatments.

**METHODS**

**EXPERIMENTAL ANIMALS**

All experiments were carried out in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Care and Use Committee of the University of Wisconsin School of Medicine and Public Health, Madison. We used a total of 12 albino female SD and 12 albino female Wistar rats (Harlan Sera-Lab, Indianapolis, Indiana). The rats were aged 12 weeks at the start of the study and were kept in our vivarium under normal cyclic light (12 hours on/off, 7 AM to 7 PM Central time) for a week prior to experimentation. The SD and Wistar rats were each divided into normal and intense cyclic light–exposed groups. Both control and treated groups were further divided into 3 subgroups each. The 3 treated subgroups of SD and Wistar rats were exposed to 12 hours of cyclic diffusely cool white 3000-lux light (36-W compact fluorescent tubes [DL 930]; Uplift Technologies Inc, Dartmouth, Nova Scotia, Canada) for 1, 3, or 6 months. The corresponding 3 control subgroups were exposed to normal cyclic light used in the animal housing facility (approximately 70 lux) for the same periods. The 12-hour light/dark cycle was used to avoid alterations in the normal physiology of the rats by disturbing the circadian rhythms set by the eye.

In the treatment groups, pairs of clear plastic cages with wire tops were kept side by side with 2 light sources, one on each side of the 2 cages (ie, 1 light source per cage and 2 cages per shelf). Before placing rats inside their respective cages and exposing them to light, light meter measurements (Thermo Fisher Scientific Inc, Pittsburgh, Pennsylvania) were taken at the bottom of each cage to ensure equal light energy exposure inside the entire cage. The distance of the light source from either side of the cage was adjusted to provide the desired reading. The control groups were kept in identical cages in a separate room under the normal cyclic vivarium lights. The light exposure of the control rats was measured in a manner identical to that of the treatment group. All rats were monitored for signs of abnormal behavior and appearance and were weighed every other week for the duration of experimentation.

**OCULAR EVALUATIONS**

At the end of 1, 3, or 6 months, dilated fundoscopy, optical coherence tomography, and angiography were carried out under anesthesia (intraperitoneal injection of 35 mg/kg of ketamine...
hydrochloride and 5 mg/kg of xylazine hydrochloride). The pupils were dilated with tropicamide, 0.5%, and phenylephrine hydrochloride. 2.3% eye drops. The topical endoscopy fundus imaging system was used to document changes in the retina following a technique previously described.35 Cirrus high-definition optical coherence tomography (Carl Zeiss Meditec, Jena, Germany) and the Heidelberg retinal angiography II system (Heidelberg Engineering, Inc, Heidelberg, Germany) were used to evaluate retinal edema and CNV using intraperitoneal injection of fluorescein sodium (60 mg/kg; Akorn Inc, Buffalo Grove, Illinois) and indocyanine green (6 mg/kg; Akorn Inc). The rat eyes were manually positioned in front of the optical coherence tomography and angiography camera to get the optimal view. The interpretation of optical coherence tomographic and angiographic images was performed by 2 masked readers (A.N. and S.W.).

HISTOLOGICAL EVALUATIONS

The rats were euthanized at 1, 3, or 6 months in a carbon dioxide chamber. The eyes were enucleated and fixed in 10% neutral-buffered formalin. Further histological processing consisted of dehydration through increased concentration of alcohol, clearing with xylene, and embedding in a horizontal axis in paraffin. Six serial pupillary–optic nerve sections and 4 non-pupillary–optic nerve anterior-posterior sections of 8-μm thickness were obtained from each eye. These sections were stained with hematoxylin-eosin, periodic acid–Schiff, or Masson trichrome stain. Comparative histopathological evaluation of the neurosensory retina, RPE, Bruch membrane, and choroid were performed in each eye by light microscopy. The retinal thickness was measured from the internal limiting membrane to the outer limiting membrane. Measurements were taken at 4 corresponding areas (2 each on the nasal and temporal sides of the optic disc). These areas were located 0.5 mm and 1 mm from the optic disc. The average retinal thickness was calculated for each treated and control rat after 1, 3, or 6 months of light exposure.

IMMUNOFLUORESCENCE STAINING FOR OXIDATIVE STRESS–RELATED PRODUCTS

The extent of oxidative stress in light-exposed and control rats was determined from the level of modifications of proteins by HNE and nitrotyrosine using immunofluorescence staining of eye sections.36 Paraffin eye sections, prepared as described, were washed in xylene 4 times (5 minutes each), followed by 2 washes with 100% and 95% ethanol (10 minutes each), respectively. After two 5-minute washes in water, sections were heated in a microwave for 11 minutes for antigen retrieval (1600 mL of water and 15 mL of vector H-3300; Vector Laboratories, Burlingame, California) and allowed to cool to room temperature overnight. Sections were then washed in water for 5 minutes, washed in phosphate-buffered saline (PBS) 3 times (5 minutes each), and incubated in blocker solution (1% bovine serum albumin, 0.2% skim milk, and 0.3% Triton X-100 in PBS) for 15 minutes at room temperature. The blocked sections were then incubated with rabbit anti–HNE antiserum (Alpha Diagnostic International Inc, San Antonio, Texas), rabbit antinitrotyrosine (Invitrogen Corp, Carlsbad, California), rabbit anti-proliferating cell nuclear antigen (Sigma-Aldrich Co, St Louis, Missouri), rabbit anti-Ki67 (Dako Corp, Carpinteria, California), or rabbit IgG (Sigma-Aldrich Co) (all prepared in blocking solution at 1:500 dilution) overnight at 4°C. After 3 washes with PBS (5 minutes each), sections were incubated with secondary antibody Alexa 594 goat–antirabbit (Invitrogen Corp; 1:500 dilution prepared in blocking solution). Sections were washed 3 times with PBS, covered with PBS/glycerol (20 vol/vol), and mounted with a coverslip. Eye sections were viewed by fluorescence microscopy, and images were captured in digital format using a Zeiss microscope (Carl Zeiss Optical Inc, Chester, Virginia).

Table 1. Distinct Histological Features of Wistar and Sprague-Dawley Rats After 1, 3, or 6 Months of Cyclic Light Exposure

<table>
<thead>
<tr>
<th>Duration of Cyclic Light Exposure in Treated Rats</th>
<th>Changes Observed in All Light-Exposed Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neural Retina</td>
</tr>
<tr>
<td>1 mo</td>
<td>Total loss of inner and outer segments; outer nuclear layer reduced to less than one-third of original thickness</td>
</tr>
<tr>
<td>SD</td>
<td>Same as Wistar rats</td>
</tr>
<tr>
<td></td>
<td>RPE Layer</td>
</tr>
<tr>
<td>1 mo</td>
<td>A few focal areas of proliferating RPE cells</td>
</tr>
<tr>
<td>SD</td>
<td>Same as Wistar rats</td>
</tr>
<tr>
<td></td>
<td>Early formation of sub-RPE neovascularization</td>
</tr>
<tr>
<td></td>
<td>Intact</td>
</tr>
<tr>
<td>3 mo</td>
<td>Total loss of inner and outer segments and outer nuclear layer disrupted by invading fibrotic bands containing blood vessels</td>
</tr>
<tr>
<td>SD</td>
<td>Same as Wistar rats</td>
</tr>
<tr>
<td></td>
<td>Multiple areas of proliferating RPE cells</td>
</tr>
<tr>
<td>3 mo</td>
<td>Fewer areas of proliferating cells compared with Wistar rats</td>
</tr>
<tr>
<td>SD</td>
<td>Only a few areas of sub-RPE neovascularization observed</td>
</tr>
<tr>
<td></td>
<td>Breached in areas with proliferation of choroidal vessels</td>
</tr>
<tr>
<td>6 mo</td>
<td>Total loss of inner and outer segments and outer nuclear layer</td>
</tr>
<tr>
<td></td>
<td>inner nuclear layer thinned and areas invaded by occasional fibrotic bands containing blood vessels</td>
</tr>
<tr>
<td>SD</td>
<td>Same as Wistar rats</td>
</tr>
<tr>
<td></td>
<td>RPE cells growing into overlying retina and extending to ganglion cell layer</td>
</tr>
<tr>
<td>6 mo</td>
<td>CVN formation with choroidal vessels extending to ganglion cell layer; observable decrease in thickness of choriocapillaris</td>
</tr>
<tr>
<td>SD</td>
<td>CVN formation with choroidal vessels growing toward ganglion cell layer; no appreciable decrease in choriocapillaris thickness</td>
</tr>
<tr>
<td></td>
<td>Areas of disruption observed less frequently than in Wistar rats</td>
</tr>
</tbody>
</table>

Abbreviations: CNV, choroidal neovascularization; PAS, periodic acid–Schiff; RPE, retinal pigment epithelium; SD, Sprague-Dawley.
RESULTS

GENERAL HEALTH AND
OPHTHALMOSCOPIC FINDINGS OF ANIMALS

All treated and control groups of Wistar and SD rats appeared healthy after 1, 3, or 6 months of exposure to intense cyclic light. No changes in activity or eating and drinking habits were observed in either group. In addition, no significant differences in the body weight of animals were observed at any point in the intense light–exposed rats as compared with control rats (not shown). Furthermore, on fundus examination, no obvious differences were noted in the appearance of the retinas between the control and treated groups in either the Wistar or SD rats. No retinal hemorrhage or exudative changes were noted in any animal.

HISTOLOGY OF WISTAR RATS

The comparative histological changes in Wistar and SD rats are summarized in Table 1. In control Wistar rats after 1 month of light exposure (Figure 1A and D), the inner and outer neurosensory layers, RPE, Bruch membrane, and choroid were within normal limits in both peripheral (Figure 1A) and central (Figure 1D) parts of the retina. In the intense light–exposed rats, the inner and outer segments were markedly atrophic and the thickness of the ONL...
was reduced to less than one-third of that in the control Wistar rats after 1 month (Figure 1B). The effects were more severe in the central part of the retina (Figure 1E). In a few central areas, swollen RPE cells with vacuolated cytoplasm were detached from the Bruch membrane (Figures 1F). The inner retinal layers were unremarkable in appearance. We did not observe migration of retinal capillaries toward the RPE layer at this time.

After 3 months of light exposure, microscopic examination of the control Wistar rats (Figure 2A) revealed normal morphology of the retinal layers and choroid. In the treated group (Figure 2B), there was almost total loss of the ONL. We observed growth of new vessels extending from the choroid, penetrating the Bruch membrane, and invading the neurosensory retina. Migrating RPE cells encroached on the outer retinal layers, surrounding the new choroidal vessels (Figure 2C). We did not observe any retinal capillaries migrating toward the RPE layer and RPE neovascularization. In addition, proliferating cell nuclear antigen or Ki67 staining of sections from rats exposed to intense cyclic light for 1 month or 3 months showed no significant difference in RPE cell proliferation in intense light–exposed animals.

After 6 months of light exposure, the RPE layer in the control groups was unremarkable with a normal anatomical arrangement of the overlying retinal layers...
The intense cyclic light–exposed SD rats exhibited pathological changes in the retina, Bruch membrane, and choroid similar to those described in the Wistar rats (Figure 5). However, these changes developed more slowly and were less severe in the light-exposed SD rats compared with Wistar rats (Table 1). The foci of sub-RPE neovascularization were first evident after 3 months of light exposure in the SD rats compared with Wistar rats, in which the foci of sub-RPE neovascularization were present after 1 month of light exposure. Retinal thickness measurements and immunostaining results (HNE and nitrotyrosine) were similar to those observed in Wistar rats (not shown). In addition, no RPE neovascularization arising from the retinal capillaries was observed at the times studied (Figure 5).

HISTOLOGY OF SD RATS

The intense cyclic light–exposed SD rats exhibited pathological changes in the retina, Bruch membrane, and choroid similar to those described in the Wistar rats (Figure 5). However, these changes developed more slowly and were less severe in the light-exposed SD rats compared with Wistar rats (Table 1). The foci of sub-RPE neovascularization were first evident after 3 months of light exposure in the SD rats compared with Wistar rats, in which the foci of sub-RPE neovascularization were present after 1 month of light exposure. Retinal thickness measurements and immunostaining results (HNE and nitrotyrosine) were similar to those observed in Wistar rats (not shown). In addition, no RPE neovascularization arising from the retinal capillaries was observed at the times studied (Figure 5).
After 6 months of intense light exposure, indocyanine green (ICG) angiography in the Wistar rats showed early peripapillary hyperfluorescence that increased in a late phase, suggestive of prominent CNV (Figure 7A and B). Fluorescein angiography (Figure 7C and D) demonstrated multiple areas of CNV with hyperfluorescence suggestive of surrounding leakage. Additionally, hypopigmented areas invaded by leaking new retinal vessels were observed. In contrast, the control group did not show any foci of neovascularization or leakage on either ICG (Figure 7E) or fluorescein (Figure 7F) angiography.

The leakage of dye from the new vessels is suggestive of their prematurity and thus pathological neovascularization. Fluorescein angiography showed intraretinal neovascular networks and marked leakage from the proliferating new retinal vessels. The peripapillary CNV was more prominently observed in ICG angiography. The ICG dye can delineate choroidal vasculature better than fluorescein dye can. These leakage foci represent the histologically seen proliferating new choroidal vessels encroaching on the overlying retinal layers. Choroidal neovascular membranes often are poorly defined on fluorescein angiography because of rapid or indistinct fluorescein leakage or because of blockage of hyperfluorescence by overlying hemorrhage, lipid, turbid fluid, or pigment. Compared with fluorescein, ICG improved visualization of the choroidal circulation and enhanced visualization of some membranes that were poorly defined with fluorescein. A similar result was noted in the SD rats (not shown).

This study demonstrates the progressive stages of intense cyclic light–induced retinal degeneration in albino Wistar and SD rats. The pathological process progressed from (1) an early stage of photoreceptor loss, partial ONL loss, and alteration in the RPE layer to (2) total loss of the ONL and partial loss of the inner nuclear layer to (3) sub-RPE neovascularization, disruption of the Bruch membrane, and finally anastomosis of newly formed choroidal vessels with retinal capillaries in the outer retina. This study demonstrates the progression to an advanced stage that to our knowledge has not been previously described. These progressive pathological changes simulate the growth patterns of human CNV as described by Grossniklaus and Green. These investigators observed 3 patterns of new vessel formation on histological examination: sub-RPE, subretinal, and combined growth. Furthermore, the features described here are similar to those described in the published histological reports of human cadaver eyes with CNV. Thus, we believe that the intense cyclic light–induced CNV model described in our study closely resembles the progressive human pathological findings and accordingly may be a useful model for the study and treatment of human exudative AMD.

The development of neovascularization is a critical component of the pathogenesis of human exudative AMD resulting in visual loss. If left untreated, these new vessels result in intraretinal and/or subretinal hemorrhages and exudates, leading to destruction of viable retinal cells. The inhibition of CNV and new vessel growth within the retina by vascular endothelial growth factor antagonists has been an important milestone in the treatment of exudative AMD in patients. However, signifi-

**OPTICAL COHERENCE TOMOGRAPHY**

Areas of higher reflectivity were seen extending through the normal retina from the RPE and choroid in both the Wistar and SD rats exposed to intense light for 6 months but not in the control unexposed groups (Figure 6). The retina was thinned with loss of inner and outer segments of photoreceptors. The RPE interface showed more irregularity in the light-treated animals than in the control groups. Hyporeflective areas were present within the retina, suggestive of leakage of fluid from microvasculature of CNV in the treated groups (Figure 6B). The presence of fluid signifies that the proliferating vessels are leaky and thus abnormally forming, as occurs in humans with exudative AMD. These changes were less marked after 1 or 3 months of light exposure (not shown). The retinal thickness measurements are shown in Figure 6C. The average retinal thickness in light-exposed Wistar rats was reduced to about 90 µm after 1 month, 70 µm after 3 months, and 50 µm after 6 months. In contrast, the average retinal thickness in the control groups remained stable at about 140 µm after 1, 3, and 6 months.

**INDOCYANINE GREEN AND FLUORESCEIN ANGIOGRAPHY**

This study demonstrates the progressive stages of intense cyclic light–induced retinal degeneration in albino Wistar and SD rats. The pathological process progressed from (1) an early stage of photoreceptor loss, partial ONL loss, and alteration in the RPE layer to (2) total loss of the ONL and partial loss of the inner nuclear layer to (3) sub-RPE neovascularization, disruption of the Bruch membrane, and finally anastomosis of newly formed choroidal vessels with retinal capillaries in the outer retina. This study demonstrates the progression to an advanced stage that to our knowledge has not been previously described. These progressive pathological changes simulate the growth patterns of human CNV as described by Grossniklaus and Green. These investigators observed 3 patterns of new vessel formation on histological examination: sub-RPE, subretinal, and combined growth. Furthermore, the features described here are similar to those described in the published histological reports of human cadaver eyes with CNV. Thus, we believe that the intense cyclic light–induced CNV model described in our study closely resembles the progressive human pathological findings and accordingly may be a useful model for the study and treatment of human exudative AMD.

The development of neovascularization is a critical component of the pathogenesis of human exudative AMD resulting in visual loss. If left untreated, these new vessels result in intraretinal and/or subretinal hemorrhages and exudates, leading to destruction of viable retinal cells. The inhibition of CNV and new vessel growth within the retina by vascular endothelial growth factor antagonists has been an important milestone in the treatment of exudative AMD in patients. However, signifi-
cant improvement of vision has been observed in only 30% of patients, and 20% of patients had a decrease in visual acuity resulting in legal blindness. In addition, potential systemic ill effects following continuous inhibition of vascular endothelial growth factor at sites necessary for normal functions, such as the retina and renal glomeruli, are of increasing concern. Thus, the development of additional treatment strategies with more specific targeting of the pathological findings associated with exudative AMD is desirable.

Various animal models have been instrumental in advancing our understanding of both the pathogenesis of AMD and the development and testing of effective treatments for AMD. An early and important animal model was the laser-induced CNV developed by Ryan in 1979, which remains one of the commonly used models for exudative AMD research. This was followed by numerous transgenic models and surgically injected growth factor–induced CNV models. A comparison of the model described here with other major CNV models is presented in Table 2. Among the available transgenic models, development of CNV was shown in the Ccl2(−/−)- and Ccr2(−/−)-deficient models, very low-density lipoprotein receptor mouse strain, hephaestin and ceruloplasmin double-knockout mice.

![Figure 6](https://jamanetwork.com/)

**Figure 6.** Representative images from optical coherence tomographic evaluation of Wistar rats after 6 months of intense cyclic light exposure and average retinal thickness measurements for Wistar and Sprague-Dawley (SD) rats. A, Retinal optical coherence tomographic scan from control Wistar rats showed that all of the layers of retina are intact. B, Retinal optical coherence tomographic scan from intense light–exposed Wistar rats showed the thinned retina with loss of photoreceptors and complete outer and partial inner nuclear layers. Hyporeflective intraretinal microcystic spaces (arrows) were also present. Similar patterns were observed in rats exposed to intense light after 1 or 3 months but to a lesser extent (not shown). C, The average retinal thickness measurements from the internal limiting membrane to the outer limiting membrane are shown. A significant decrease in the average retinal thickness in the light-exposed Wistar and SD rats was observed after 1, 3, or 6 months of intense light exposure when compared with the control groups (P<.05; n=3 in each subgroup). Error bars indicate standard error of the mean.

![Figure 7](https://jamanetwork.com/)

**Figure 7.** Representative images from indocyanine green angiography and fluorescence angiography of Wistar rats after 6 months of intense cyclic light exposure. Early-phase indocyanine green angiography showed mild peripapillary hyperfluorescence (A) that increased further in a late phase (B), suggestive of peripapillary choroidal neovascularization (arrows). C and D, Intraretinal anastomosing new vessels, hypoperfused areas, and numerous focal hyperfluorescence areas of choroidal neovascularization (arrows) were seen on fluorescein angiography. Compared with fluorescein, indocyanine green improved visualization of choroidal circulation and enhanced visualization of some membranes that were poorly defined with fluorescein. In control groups, there were no foci of neovascularization or leakage on indocyanine green (E) and fluorescein (F) angiography. All animals exposed to intense cyclic light exhibited a similar phenotype.
model, and SoD1-deficient mice. However, these pathological changes were observed in only significantly older animals (aged 10-15 months). In addition, the corresponding genes causing the retinal changes in these models have not been associated directly with AMD in humans. Thus, availability of models that develop the entire spectrum of AMD-associated pathological changes within a reasonably short duration would be extremely useful in the study of the disease as well as in the development and testing of new targeted treatments.

Oxidative stress is one of the pathological processes recognized to be involved in human AMD. The increased oxidative stress–induced by-products HNE and nitrotyrosine in the degenerated retina with light–induced oxidative stress–induced by-products HNE and nitrotyrosine in the degenerated retina with light-induced CNV were observed in our model, as previously reported in various animal models using light as the stimulus, Cousins et al. Among models showing light as the stimulus, Cousins et al. reported the presence of basal laminar deposits with excessive fatty diets; these deposits increased in severity with brief exposure to blue light. In contrast, the rats in our study were fed a normal diet but exposed to a prolonged duration of 3000-lux cyclic white light and developed sub-RPE deposition of amorphous material similar to basal laminar deposits described in the literature.

A focal photic injury had been used to stimulate CNV in the past but never in association with retinal degeneration related to light exposure. Although neovascularization has been previously reported in various animal models of retinal degeneration, most of these cases had an origin from retinal capillaries and extended to the RPE and choroid, resulting in RPE and choroid atrophy. However, the effect on RPE cells was also minimal in these animals. These observations are consistent with those reported in our study in rats exposed to intense cyclic light, where extensive loss of photoreceptor cells was observed by the first month. This was concomitant with the migration of newly formed choroidal—not retinal—blood vessels growing toward the outer retina. The RPE cells appeared, for the most part, within normal limits at 1 and 3 months of intense light exposure with minimal signs of proliferation determined by proliferating cell nuclear antigen or Ki67 staining (not shown). However, loss of RPE polarization and organization of RPE

---

**Table 2. Comparison of the Light-Induced Model With Existing Transgenic and Surgically Intervened Animal Models Producing Retinal or Choroidal Neovascularization**

<table>
<thead>
<tr>
<th>Name</th>
<th>Genetic Modification or Surgical Implantation</th>
<th>Features</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccl2 (−/−)</td>
<td>Ccl2 (−/−) knockout mouse (MCP1)</td>
<td>Lipofuscin, A2E, BLD, drusen, PR atrophy, CNV</td>
<td>Ambati et al., 2003; Hahn et al., 2004; Lu et al., 1998; Kuziel et al., 1997</td>
</tr>
<tr>
<td>Ccr2 (−/−)</td>
<td>Ccr2 (−/−) knockout mouse (Ccr2)</td>
<td>PR and RPE atrophy, CNV</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Cpr(−/−)Hep(−/+)</td>
<td>Cpr knockout mouse (Gp) crossed with spontaneous sla mutation in C57BL/6</td>
<td></td>
<td>------------------------------</td>
</tr>
<tr>
<td>r/hOta-TRE/VEGF</td>
<td>Transgenic, rhodopsin promoter driving inducible human VEGF expression</td>
<td></td>
<td>------------------------------</td>
</tr>
<tr>
<td>IRBP/rOta-TRE/VEGF</td>
<td>Transgenic, IRBP promoter driving inducible human VEGF expression</td>
<td>Retinal neovascularization</td>
<td>Obata et al., 1996</td>
</tr>
<tr>
<td>RPE65/VEGF</td>
<td>Transgenic, RPE65 promoter driving expression of murine VEGF</td>
<td>Intraocular neovascularization</td>
<td>Schwersinger et al., 2001</td>
</tr>
<tr>
<td>VLDLR(Vdmltm1Her)</td>
<td>Vdml (−/−) knockout mouse (Vdml)</td>
<td>Choroidal anastomosis, retinal neovascularization</td>
<td>Frykman et al., 1995; Heckenlively et al., 2003</td>
</tr>
<tr>
<td>Matrigel model</td>
<td>Subretinal injection of Matrigel</td>
<td>RPE vacuolization and migration, inflammatory cell infiltrations within the Matrigel deposit, CNV</td>
<td>Shen et al., 2006; Zhao et al., 2007</td>
</tr>
<tr>
<td>bFGF rabbit model</td>
<td>Subretinally implanted bFGF-impregnated gelatin microspheres</td>
<td>Subretinal neovascularization in 83% of their rabbit eyes</td>
<td>Kimura et al., 1995</td>
</tr>
<tr>
<td>VEGF pellet implantation model</td>
<td>Intravitreous injection of VEGF pellets</td>
<td>Transient retinal neovascularization</td>
<td>Ozaki et al., 1997</td>
</tr>
<tr>
<td>Light-induced rat model</td>
<td>No genetic or surgical manipulation, long-term cyclic high-intensity light exposure</td>
<td></td>
<td>------------------------------</td>
</tr>
</tbody>
</table>

Abbreviations: A2E, N-retinylidene-N-retinylethanolamine; bFGF, basic fibroblast growth factor; BLD, basal laminar linear deposits; Ccl2, chemokine (C-C motif) ligand 2; Ccr2, C-C chemokine receptor-2; CNV, choroidal neovascularization; Cpr, ceruloplasmin; Hep, hephestin; IRBP, interphotoreceptor retinoid binding protein; MCP1, monocyte chemoattractant protein 1; PR, photoreceptor; rho, rhodopsin; RPE, retinal pigment epithelium; VEGF, vascular endothelial growth factor; VLDLR, very low-density lipoprotein receptor.

*Adapted from the study by Elizabeth Rakoczy et al.*
cells around the newly forming choroidal blood vessels were observed. Thus, our results suggest that the rapid loss of photoreceptor cells may promote the formation of new choroidal vessels to accommodate the oxygen need of the outer retina.

Neovascularization of the RPE by retinal capillaries is a common feature of inherited and environmentally induced retinal degeneration in rodents. However, neovascularization of the RPE is not known to occur in human diseases of photoreceptor degeneration such as retinitis pigmentosa. Although it is suggested that other types of neovascularization may occur, their source and pathogenesis remain elusive. Furthermore, the studies in different rodent models of retinal degeneration suggest that a major contributing factor may be temporal (i.e., the rate at which photoreceptors degenerate) and thus determines the involvement of retinal or choroidal capillaries as the source of vascularization of the outer retina. Therefore, the gradual loss of photoreceptor cells over time may favor migration of retinal capillaries with consequent RPE neovascularization. The molecular and cellular mechanisms that discriminate between these pathways remain elusive and need further study.

In summary, our rat model displayed progressive stages of retinal degeneration, with features of newly forming choroidal vessels closely resembling those seen in human exudative AMD, induced by long-term intense cyclic light exposure within a relatively short time. We believe the long-term intense cyclic light–induced exudative AMD model described here will prove useful not only in the evaluation of molecular and cellular mechanisms associated with exudative AMD but also for effective screening of new compounds with therapeutic potential.

Submitted for Publication: August 13, 2009; final revision received November 19, 2009; accepted November 20, 2009.

Correspondence: Nader Sheibani, PhD, Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, 600 Highland Ave, K6/458 CSC, Madison, WI 53792-4673 (nsheibanikar@wisc.edu).

Financial Disclosure: None reported.

Funding/Support: This work was supported in part by grants EY16995 (Dr Sheibani), EY18179 (Dr Sheibani), and P30 EY16665 from the National Institutes of Health and an unrestricted departmental award from Research to Prevent Blindness. Dr Sheibani is a recipient of a research award from the Retina Research Foundation.

Additional Contributions: Jaal B. Ghandhi, PhD, College of Engineering, University of Wisconsin, Madison, assisted with the design and measurement of the intense cyclic lighting. Elizabeth A. Scheef, MS, prepared the figures, and Carol Rasmussen, BS, helped with optical coherence tomography, ICG angiography, and fluorescein angiography.

REFERENCES


