A Mouse Model of Retinal Recovery From Photo-Oxidative/Photo-Inflammatory Injury: Nrf2, SOD1, DJ-1, and Parkin Are Not Essential to Recovery

Bo Chen,¹,² Bogale Aredo,¹ Yuanfei Zhu,*,¹ Yi Ding,†,¹ Cynthia Xin-Zhao,¹ and Rafael L. Ufret-Vincenty¹

¹Department of Ophthalmology, UT Southwestern Medical Center, Dallas, Texas, United States
²Permanent Address: Department of Ophthalmology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei Province, China

Correspondence: Rafael L. Ufret-Vincenty, Department of Ophthalmology, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9057, USA; Rafael.Ufret-Vincenty@UTSouthwestern.edu.

BC, BA, and YZ contributed equally to the work presented here and should therefore be regarded as equivalent authors.

Current Affiliation: †Department of Ophthalmology, Shenzhen Key Laboratory of Ophthalmology, Shenzhen University School of Medicine, Shenzhen, Guangdong, China.

Current Affiliation: ‡Department of Ophthalmology, Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR. China.

Submitted: September 20, 2018
Accepted: February 8, 2019

Citation: Chen B, Aredo B, Zhu Y, Ding Y, Xin-Zhao C, Ufret-Vincenty RL. A mouse model of retinal recovery from photo-oxidative/photo-inflammatory injury: Nrf2, SOD1, DJ-1, and Parkin are not essential to recovery. Invest Ophthalmol Vis Sci. 2019;60:1165–1174. https://doi.org/10.1167/iovs.18-25751

PURPOSE. To determine if there is structural and functional recovery of the retina from light induced retinal degeneration, and to evaluate the role of the oxidative stress response elements Nrf2, SOD1, DJ-1, and Parkin in such a recovery process.

METHODS. Eyes from C57BL/6J (B6J) mice and from oxidative stress response-deficient strains of mice were treated with intense light using the fundus camera-delivered light-induced retinal degeneration (FCD-LIRD) model. Fundus photographs, optical coherence tomography (OCT) images, and electroretinography (ERG) responses were obtained before the injury, during the “maximal injury phase” (days 4–7) and during the “recovery phase” (days 14–16) post light exposure and were evaluated for retinal damage and assessed for evidence of recovery from the injury.

RESULTS. We demonstrate that mice treated with a sub-lethal FCD-LIRD protocol show an initial acute retina injury phase peaking between days 4 to 7 followed by a recovery phase in which the outer retinal thickness/volume and retinal function partially recover. These observations are reproduced in B6J mice and in mice lacking oxidative stress response enzymes (SOD1, DJ-1, and Parkin) or the oxidative stress response master regulator Nrf2.

CONCLUSIONS. Our data indicate that retinal recovery from injury can proceed via pathways that are independent from the common oxidative stress response elements Nrf2, SOD1, DJ-1, and Parkin. Furthermore, the model of retinal recovery from injury that we describe here mimics changes seen in a variety of clinical entities and may provide an excellent platform for dissecting general pathways of retinal recovery from sub-lethal injury.

Keywords: light-induced retinal degeneration, oxidative stress, retinal recovery, Nrf2, FCD-LIRD

A great deal of interest has been devoted to the important topic of understanding the mechanisms of acute retinal injury and developing strategies to protect photoreceptors from acute injury.¹⁻² However, in many human retinal diseases, the damage involves repetitive sub-lethal stressors. In fact, by the time of diagnosis, such damage has often already started or may be difficult to completely avoid. Understanding how the retina recovers from repetitive or persistent low-level injury may give us the information needed to optimize the speed and the extent to which the photoreceptors and RPE overcome insults.

Multiple human clinical studies show that photoreceptors and RPE cells can partially recover after injury and that residual abnormalities may account for suboptimal clinical outcomes.¹⁰⁻²⁰ These studies cover a wide range of retinal diseases with damage to the photoreceptors and RPE, including central serous retinopathy,¹⁰⁻¹² phototoxity,¹³,¹⁴ retinal detachment,¹⁵⁻¹⁷ inflammatory diseases,¹⁸,¹⁹ and even AMD.²⁰ They document a clinical course characterized by initial anomalies of the photoreceptor outer segments on OCT, followed by partial recovery. If recovery is insufficient, this leads to vision loss. Similar clinical and OCT findings have been described in non-human primates after light-induced macular damage.²¹⁻²³ Interestingly, using adaptive optics scanning light ophthalmoscopy imaging, Scoles et al.²⁴ showed that they could detect photoreceptors in areas of ellipsoid zone disruption after blunt ocular trauma and retinal dystrophies and wondered about ways of restoring function to these cells. While a few in vitro
studies have shown that ARPE-19 cells are able to recover to some extent from sub-lethal oxidative injury.\textsuperscript{25,26} The investigators have concluded that more work needs to be devoted to understanding the mechanisms, particularly using in vivo models.

To study this exciting idea, an appropriate test system is needed. We have recently developed a new mouse model of light-induced retinal degeneration that is mediated by oxidative stress/inflammation and easily modulated in terms of severity.\textsuperscript{27,28} This fundus camera-delivered light-induced retinal degeneration (FCD-LIRD) model is a photo-oxidative/photo-inflammatory injury model, effective in mice carrying the RPE65 450-Met variant and thus can be used in C57BL/6j (B6j) mice and genetically manipulated mice on that background.

We will refer to this model as “photo-oxidative/photo-inflammatory” because oxidative stress and inflammatory process may be interrelated in this model, as they are in multiple human diseases. We show that there are aspects of the model that are consistent with a photo-inflammatory mechanism, including the accumulation of subretinal microglia and the accumulation of C3d in the subretinal space.\textsuperscript{27} On the other hand, some aspects that point toward a photo-oxidative mechanism include: (1) the fact that the injury is more severe in animals deficient in oxidative stress response enzymes\textsuperscript{29,30} or oxidative stress master regulator Nrf2, and (2) that we have shown that, after FCD-LIRD, there is increased subretinal accumulation of hemoxygenase-1,\textsuperscript{27} a molecule commonly used as a marker of oxidative stress. Finally, we have documented an increase in multiple genes related both to inflammation and oxidative stress, including \textit{HO-1}, \textit{Srxn1}, \textit{Cebpd}, \textit{Gadd45b}, \textit{Ppard}, and \textit{Socs3}.\textsuperscript{27,28} We believe that this combination of oxidative stress and inflammatory components makes this model more relevant to human disease.

In the current work, we demonstrate that in mice treated with a sub-lethal FCD-LIRD protocol, an initial acute retina injury phase leads to changes in the reflectivity and thinning of the outer retina on OCT, and a subsequent recovery phase in which the retinal volume between Bruch’s membrane and the RPE65 450-Met variant and thus can be used in C57BL/6j (B6j) mice and genetically manipulated mice on that background.

We refer to this model as “photo-oxidative/photo-inflammatory” because oxidative stress and inflammatory process may be interrelated in this model, as they are in multiple human diseases. We show that there are aspects of the model that are consistent with a photo-inflammatory mechanism, including the accumulation of subretinal microglia and the accumulation of C3d in the subretinal space.\textsuperscript{27} On the other hand, some aspects that point toward a photo-oxidative mechanism include: (1) the fact that the injury is more severe in animals deficient in oxidative stress response enzymes\textsuperscript{29,30} or oxidative stress master regulator Nrf2, and (2) that we have shown that, after FCD-LIRD, there is increased subretinal accumulation of hemoxygenase-1,\textsuperscript{27} a molecule commonly used as a marker of oxidative stress. Finally, we have documented an increase in multiple genes related both to inflammation and oxidative stress, including \textit{HO-1}, \textit{Srxn1}, \textit{Cebpd}, \textit{Gadd45b}, \textit{Ppard}, and \textit{Socs3}.\textsuperscript{27,28} We believe that this combination of oxidative stress and inflammatory components makes this model more relevant to human disease.

In the current work, we demonstrate that in mice treated with a sub-lethal FCD-LIRD protocol, an initial acute retina injury phase leads to changes in the reflectivity and thinning of the outer retina on OCT, and a subsequent recovery phase in which the retinal volume between Bruch’s membrane and the external limiting membrane partially recovers. These observations parallel similar findings described in primates. Further, while we and others have shown that the oxidative stress response is very important in the acute injury phase,\textsuperscript{1-3,27-30} we have also demonstrated that mice lacking oxidative stress response enzymes (SOD1, DJ-1, and Parkin) or the oxidative stress response master regulator Nrf2—despite suffering increased acute injury—still show significant recovery after the initial injury. This suggests that classic oxidative stress response pathways are not essential for retinal recovery after injury.

Since this model mimics very well the changes seen in a variety of clinical entities, we now have a test system that can be used to understand general pathways of retinal recovery from injury across various retinal pathologies that involve sub-lethal injury.

**METHODS**

**Animals**

Animal experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All procedures were approved by the UT Southwestern Medical Center Institutional Animal Care and Use Committee (IACUC, Protocol # 2015-0109957). Experiments with TKO mice (“triple KO mice” are simultaneously deficient in SOD1/DJ-1/Parkin) and age- and sex-matched C57BL/6j (B6j) littermates were tested as reported before.\textsuperscript{28} Nrf2 KO mice were obtained from The Jackson Laboratory (NIE212; nuclear factor, erythroid derived 2, like 2, stock no. 017009; Bar Harbor, ME, USA). These mice had been backcrossed to B6j ten times by the original investigator donating the mice to The Jackson Laboratory, and then at least once more by The Jackson Laboratory. However, the mice are still on a mixed B6j/BGN background. Thus, we backcrossed them again to B6j three additional times to ensure we eliminated the RD8 mutation of the \textit{Crbi} gene, which was checked by genotyping.\textsuperscript{31-33} Nrf2 genotyping was done using the primers and protocol recommended by The Jackson Laboratory: Nrf2 common_forward (5’-GGCTAGAGGCTG TAGGGCC-3’), Nrf2 WT_reverse (5’-GGAATGAAAA TAGCCTCGCC-3’), and Nrf2 Mut_reverse (5’-GACAG TATCGGCCTCAGGAA-3’). For our experiments Nrf2 KO and control B6 mice were bred and kept in a barrier animal facility at UTSW under normal lighting conditions with 12-hour-on/12-hour-off cycles with free access to food and water. Before performing all procedures, mice were anesthetized with a ketamine-xylazine cocktail (100mg/kg-5mg/kg) one at a time.

Mouse eyes were dilated using one drop per eye of a mixture (1:1) of tropicamide 1% solution (Alcon Laboratories, Inc., Fort Worth, TX, USA) and phenylephrine hydrochloride 2.5% solution (Alcon, Inc., Lake Forest, IL, USA).

**Fundus Photography**

Fundus photographs of mice were obtained using a Micron IV mouse fundus camera (Phoenix Research Laboratories, Pleasanton, CA, USA) as described before.\textsuperscript{34} Briefly, a fundus image of each eye, centered on the optic nerve head, was obtained after sharply focusing on the retinal pigment epithelium (RPE).

**Fundus-Camera Derived Light-Induced Retinal Degeneration (FCD-LIRD) Model**

Following overnight dark adaptation, each mouse was anesthetized and the pupils dilated. Light intensity from the Micron IV mouse fundus camera was measured using a light meter (Cat # S90199; Fisher Scientific, Pittsburgh, PA, USA) to ensure that equal illumination was provided to all eyes. Using the Micron IV fundus camera, we applied light to the retina, centered on the optic disc, following the FCD-LIRD protocols as described before.\textsuperscript{27} For the mild “light-only” model of light injury, the Micron IV fundus camera was focused on the RPE layer, and light was applied to the retina at an intensity of 95 K lux or 100 K lux (depending of the desired severity of the light injury for the specific experiment) for a one-time exposure of 30 minutes. For the moderate fluorescein-assisted model, fluorescein was administered as a single intraperitoneal injection of 100 µl of a 1.5 dilution of commercially available 10% fluorescein solution (total dose of 2 mg of fluorescein). Light was then applied to one eye at an intensity of 50 K lux or 54 K lux (see Figure legends for individual experiments) for a duration of 4 minutes, starting 3 minutes after the fluorescein injection (Fl-3@3). For the more severe fluorescein assisted model, the eye received 45 K lux of light for 3 minutes, starting 10 minutes after the fluorescein injection (Fl-3@10). Mice were kept under normal lighting conditions after the procedure.

**Image-Guided OCT and Retinal Layer Analyses**

Mice were anesthetized and pupils were dilated. GenTeal liquid gel (Novartis, East Hanover, NJ, USA) was applied to the corneal surface. For qualitative and quantitative retinal layer analyses, optical coherence tomography (OCT) images were
taken using an image-guided tomographer (Micron IV-OCT2; Phoenix Research Laboratories, Pleasanton, CA, USA) by placing a short (half-size) horizontal line two disc diameters superior to the optic disc. Qualitative assessment of retinal integrity was done by looking at the change in reflectivity of the outer retinal layers on OCT images. From prior studies we have defined a “maximal injury phase” (days 4–7 after light exposure). During the current study, we found evidence of retinal recovery at days 14–16 after light exposure, which we define here as the “recovery phase.” We obtained OCT images at baseline, during the maximal injury phase, and during the recovery phase. For quantitative measurement of the retinal layer thickness at the target location, we used the Freehand tool in ImageJ (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). Three measurements were taken 200 μm apart in the center of the image for two measured parameters: (1) outer retina between Bruch’s membrane and the external limiting membrane and (BM OLM) and (2) outer retina between Bruch’s membrane and the bottom of the outer plexiform layer (BM OPL). Finally, the data for each image/eye were averaged for statistical analysis.

**OCT Volume Analysis**

OCT volume was measured from a block of retinal images obtained using the Reveal/OCT2 systems (Phoenix Research Laboratories) as described before. In brief, a 3D volume image was captured in a standard square region generated by the system (approximately 5 DD × 5 DD in size) centered on the optic nerve head (ONH). The images were then opened with InSight 3D Voxeleron intelligent segmentation software for quantitative assessment of the specific outer retinal regions. Finally, the data were exported to Excel worksheets for quantitative analysis.

**OCT Grading System**

OCT images were opened in ImageJ, and a grading score from 0 to 8 was assigned by a masked investigator based on the level of damage to the retinal layers (see Supplementary Table S1).

**Electroretinogram (ERG)**

ERG responses were recorded in dark-adapted Nrf2 KO mice and age- and sex-matched B6 control mice at baseline, and at different time points post light injury (ERG was done only on one eye) using a scotopic Ganzfeld ERG system (Phoenix Research Labs) as recommended by the company. Briefly, mice were dark-adapted overnight for 16 hours. After anesthesia and pupil dilation, mice were placed on a platform covered by a homeothermic heating blanket to maintain body temperature, and preparations were made under a dim red light. GenTeal liquid gel was applied to each eye after anesthesia to prevent corneal drying and to establish contact with the lens. The reference and the ground electrodes (platinum needles) were subcutaneously inserted on the top of the head and into the tail, respectively. Scotopic Ganzfeld ERGs were obtained in response to low (0.1 log cd.s.m−2) and high (3.1 log cd.s.m−2) green light flash intensities at baseline, 4 days after light exposure (using the FCD-LIRD Fl-4@3 protocol) and 14 days after light exposure. We observed a reduction both in a-wave (Fig. 1B) and b-wave (Fig. 1C) amplitudes 4 days after the light stimulus, followed by recovery by day 14 (P values for recovery of the a-wave were 0.0005 for the low stimulus and 0.041 for the high stimulus; P values for recovery of the b-wave were 0.054 for the low stimulus and 0.042 for the high stimulus). One-way ANOVA confirmed that the difference between the three time points was statistically significant (P < 0.05) for both a-wave and b-wave (except for a-wave with high stimulus in Fig. 1C, where there is only a trend of P = 0.1). Finally, to determine if this process of retinal recovery was affected by the oxidative stress response, we re-analyzed our data on FCD-LIRD experiments using mice simultaneously deficient in the anti-oxidant defense proteins SOD1, DJ-1, and Parkin (TKO mice). These three proteins are shown to be active in the retina and in regulating oxidative stress in the retinal environment. We had previously shown that these TKO mice have increased susceptibility to acute retinal injury compared to B6 control mice. We now found that the TKO mice also showed retinal recovery after the initial FCD-LIRD injury, despite the absence of these three antioxidant proteins (Fig. 2). Using ANOVA followed by Tukey’s post-hoc test was used to analyze data involving three time points. A 2-way ANOVA followed by Tukey’s post-hoc test was used to compare data in which the two independent variables of time (baseline, day 5, and day 15) and treatment protocol (Light only versus Fl-4@3) were included. A P value < 0.05 was considered statistically significant.

**Results**

**Structural and Functional Recovery of the Retina From Light Damage in C57BL/6 Mice**

Using the FCD-LIRD model as an exogenous photo-oxidative/photo-inflammatory stress stimulus, we had previously shown that B6 control retinas demonstrated significant damage at day 5 post-injury compared to baseline. We now performed additional analysis of our to determine the clinical course of this injury. When we compared the OCT outer retinal volume 15 days after injury to that seen 5 days after injury, we found that there was a statistically significant recovery in outer retinal volume by day 15 after injury (Fig. 1A, P = 0.0075). One-way ANOVA confirmed a difference when comparing the three time points (P < 0.005). We decided to explore this further by reproducing the experiment, this time looking at a functional outcome to determine if the structural recovery seen on OCT translated to a functional recovery. Scotopic Ganzfeld ERGs were obtained in B6j mice in response to low (0.1 log cd.s.m-2) and high (3.1 log cd.s.m-2) green light flash intensities at baseline, 4 days after light exposure (using the FCD-LIRD Fl-4@3 protocol) and 14 days after light exposure. A two-tailed Student’s t-test or the Mann-Whitney U test was performed when comparing two groups. One-way ANOVA followed by Tukey’s post-hoc test was used to analyze data involving three time points. A 2-way ANOVA followed by Tukey’s post-hoc test was used to compare data in which the two independent variables of time (baseline, day 5, and day 15) and treatment protocol (Light only versus Fl-4@3) were included. A P value < 0.05 was considered statistically significant.
Retinal Recovery is Evidenced Clinically in Fundus Images and OCT in B6J and Nrf2 KO Mice

Based on the observations in B6J and TKO mice, we decided to further explore the phenomenon of retinal recovery after FCD-LIRD—in particular, to explore if it was affected by a deficit in the oxidative stress response pathways. The importance of Nrf2 as a master regulator of the oxidative stress response in the retina has been demonstrated before. Thus, we obtained fundus photos and OCT images of B6J mice and Nrf2 KO mice at baseline, day 5, and day 15 after a moderate injury based on the FCD-LIRD protocol (Fl-4@3). At day 5, we found we could clearly observe retinal damage in the fundus photos and OCT images of both Nrf2 KO mice (Figs. 3E and 3K vs. 3D and 3J) and B6J mice (Figs. 3B and 3H vs. 3A and 3G). Furthermore, as we predicted based on prior observations that FCD-LIRD was oxidative-stress-mediated, we found that the damage was more severe in Nrf2 KO compared to B6J mice (Figs. 3E vs. 3B, and 3K vs. 3H; Supplementary Fig. S1). However, even in the absence of the oxidative stress response master regulator Nrf2, we did observe a partial recovery in both fundus photos and OCT images in Nrf2 KO mice (Figs. 3F vs. 3E, and 3L vs. 3K). As indicated by the white arrows, the hyper-reflectivity of the ellipsoid zone is diminished 5 days after the light exposure compared to baseline in both B6J and Nrf2 KO mice (3H vs. 3G, and 3K vs. 3J, respectively). Concomitantly, the underlying hyporeflective outer segment band seems to disappear, and the interdigitation zone becomes hyporeflective. OCT images taken in the same location at day 15 post-injury show a partial recovery of the hyperreflectivity of ellipsoid and interdigitation zones and a partial recovery of
the hyporeflective outer segment band in both groups (3I vs. 3H, and 3L vs. 3K).

Grading and Quantification of Retinal Damage in Nrf2 KO Versus B6J Using OCT Image

We developed an OCT-based retinal injury grading system (see Methods and Supplementary Table S1) for a semi-quantitative analysis of the structural integrity of the retinal layers in FCD-LIRD-treated eyes compared to naïve/uninjured eyes. This grading system takes into consideration qualitative changes in the images (change in reflectivity and outer retinal disruption) that go beyond retinal thickness changes. Nrf2 KO mice showed a significant increase in retinal damage compared to B6J mice at day 5 after light exposure. This was the case with either the moderate FCD-LIRD protocol (Fl-4@3, Fig. 4A, \( P = 0.032 \)), or a more intense one (Fl-3@10, Fig. 4B, \( P = 0.0083 \)). We then measured the outer retinal thickness (from BM to the bottom of the OPL, Fig. 4C, or from BM to the ELM, Fig. 4D), and found that in addition to the qualitative and semi-quantitative changes in structural integrity, the Nrf2 KO mice demonstrated significant outer retinal thinning compared to the B6J controls (Fig. 4C, \( P = 0.014 \), and Fig. 4D, \( P = 0.046 \)).

Structural and Functional Recovery of the Nrf2 KO Retinas in Mice Exposed to Two Levels of FCD-LIRD

Having demonstrated that deficiency of the oxidative stress response master regulator Nrf2 led to increased photo-oxidative/photo-inflammatory injury to the retina, we proceeded to test whether this deficiency abrogated the retinal recovery phase after injury. To this end, we measured retinal thickness on OCT images obtained from Nrf2 KO and B6J control mice at day 5 and day 16 post–FCD-LIRD and normalized them to baseline (Fig. 5). First, we corroborated a statistically significant recovery (by day 16) of the outer retinal thickness (BM-ELM) in B6J mice both after a moderate FCD-LIRD protocol (Fl-4@3, Fig. 5A, \( P = 0.00060 \)) and a more severe protocol (Fl-3@10, Fig. 5B, \( P = 0.000055 \)). Furthermore, we found that despite a more severe retinal injury (thinner outer retina) in Nrf2 KO mice 5 days after FCD-LIRD (Fig. 5C vs. 5A and 5D vs. 5B), these mice still demonstrated statistically significant recovery by day 16. This recovery was seen both after a moderate FCD-LIRD protocol (Fl-4@3 in Fig. 5C, \( P = 0.0043 \)) and a more intense protocol (Fl-3@10 in Fig. 5D, \( P = 0.022 \)). Finally, we wanted to determine if the anatomical recovery in Nrf2 KO mice correlated with a functional retinal
recovery. Nrf2 KO mice underwent scotopic Ganzfeld ERGs 7 days after light exposure (FCD-LIRD Fl-4@3) and again 14 days after light exposure (Fig. 6). We observed a statistically significant recovery of both a-wave amplitude and b-wave amplitude by day 14 after light injury. Retinal damage was assessed qualitatively on OCT images (semi-quantitative damage score, A, B) or quantitatively by measuring retinal thickness (C, D). OCT images were graded based on the integrity of the retinal layers using a scale from 1 to 8 (see Supplementary Table S1). The Nrf2 KO mice show a greater damage score compared to B6j controls both in the high intensity Fl-3@10 protocol (A, n = 11 B6j and 12 Nrf2 KO mice) and the moderate intensity Fl-4@3 protocol (B, n = 10 B6j and 14 Nrf2 KO mice). The outer retinal thickness (“BM_OPL” or bottom of Bruch’s membrane to the bottom of the OPL), and “BM_ELM” or bottom of Bruch’s membrane to the external limiting membrane) was measured on OCT images using the Freehand tool in ImageJ (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). The outer retinal layers were significantly thinner in Nrf2 KO compared to B6j controls (C, D; n = 11 B6j and 14 Nrf2 KO mice). Each symbol represents mean values ± standard error of the mean for each day. * = P < 0.05, ** = P < 0.01.

FIGURE 4. Nrf2 KO mice have significantly more retinal damage than B6j mice at day 5 post light injury using the FCD-LIRD protocols. B6j and age- and sex-matched Nrf2 KO mice were subjected to two different fluorescein-assisted FCD-LIRD protocols (“Fl-4@3” consisted of 50 K lux of light applied for 4 minutes starting 3 minutes after i.p. fluorescein injection, while “Fl-3@10” consisted of 45 K lux of light applied for 3 minutes starting 10 minutes after i.p. fluorescein injection). At day 5 after light injury, retinal damage was assessed qualitatively on OCT images (semi-quantitative damage grade, A, B) or quantitatively by measuring retinal thickness (C, D). OCT images were graded based on the integrity of the retinal layers using a scale from 1 to 8 (see Supplementary Table S1). The Nrf2 KO mice show a greater damage score compared to B6j controls both in the high intensity Fl-3@10 protocol (A, n = 11 B6j and 12 Nrf2 KO mice) and the moderate intensity Fl-4@3 protocol (B, n = 10 B6j and 14 Nrf2 KO mice). The outer retinal thickness (“BM_OPL” or bottom of Bruch’s membrane to the bottom of the OPL) and “BM_ELM” or bottom of Bruch’s membrane to the external limiting membrane) was measured on OCT images using the Freehand tool in ImageJ (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). The outer retinal layers were significantly thinner in Nrf2 KO compared to B6j controls (C, D; n = 11 B6j and 14 Nrf2 KO mice). Each symbol represents mean values ± standard error of the mean for each day. * = P < 0.05, ** = P < 0.01.

DISCUSSION

Chronic oxidative stress and chronic inflammation are important—and likely interrelated—mechanisms of disease in neurodegenerative disorders, including those affecting the retina.51,42,44-53 In addition, repetitive sub-lethal stressors may play a major role in many human retinal diseases. Increasing our knowledge regarding the complex mechanisms involved in the recovery of the retina from these repetitive or persistent low-level injuries may allow us to minimize cell death or dysfunction. Although the etiologies and mechanisms of injury to the RPE and photoreceptors vary, it is reasonable to speculate that a common strategy focused on recovery mechanisms could be developed to maximize visual outcomes. To pursue this goal, a good model of retinal recovery is needed. We hypothesized that the FCD-LIRD model of retinal injury in mice could prove useful.

In this work, we found that by applying FCD-LIRD protocols of different intensities to B6j mice, we could easily document and quantify the level of retinal injury by days 4-7 after light exposure and, more importantly, we could also consistently document and quantify retinal recovery by days 14-16. Both...
injury and recovery were seen as qualitative changes in fundus photos and OCT but also as quantitative anatomical changes in OCT and in quantitative functional changes on ERG.

We had previously found that mice deficient in oxidative stress response enzymes (SOD1^{-/-}, DJ-1^{-/-}, and Parkin^{-/-} triple KO mice) demonstrated increased retinal injury after FCD-LIRD compared to B6 mice. In the present work, we extended this observation by showing that mice deficient in the oxidative stress response master regulator Nrf2 also show increased injury after FCD-LIRD compared to B6J mice. This is consistent with our observation that the acute injury phase after FCD-LIRD is oxidative stress/inflammation-mediated and that the physiologic oxidative stress response pathways can decrease the level of damage.

Moreover, we now describe that both TKO and Nrf2 KO mice, despite the increased level of injury, still show a subsequent retinal recovery that can be quantified by OCT and ERG. These findings suggest that the common oxidative stress response elements Nrf2, SOD1, DJ-1, and Parkin are not essential for the retina to recover from injury. We cannot generalize our results to suggest that the oxidative stress response is not needed because it is always possible that other stress response elements may be elevated in our mutant mice. Further, Nrf2 is not the only regulator of oxidative stress responses.

Others have shown that overexpression of Nrf2 may protect the retina from injury and aid in its recovery. Our results are not challenging that conclusion but merely establishing that retinal recovery can also occur in the absence of Nrf2. Combining both results, we postulate that retinal recovery involves multiple pathways, including some that are Nrf2-mediated and some that are Nrf2-independent. We propose that understanding these response mechanisms better may allow us to maximize retinal recovery after injury.

In summary, we now have an efficient, reliable, and quantifiable model of photo-oxidative/photo-inflammatory retinal damage and recovery that will allow us to study the cellular and molecular mechanisms of retinal recovery from injury. Furthermore, we can apply this model to genetically modified mice to explore the effect of specific genes in the process. Finally, the intensity of the model can be modified to generate a moderate level of injury, which in different genetic backgrounds may require different intensities of light exposure.

**Figure 5.** Both B6J and Nrf2 KO mice show significant outer retinal layer recovery from light damage 2 weeks after light injury with the FCD-LIRD protocols. Two separate fluorescein-assisted protocols of FCD-LIRD were applied to B6J and Nrf2 KO mice. The moderate “Fl-4@3” consisted of 50 K lux of light applied for 4 minutes, starting 3 minutes after i.p. fluorescein injection (A, n = 11 B6J; C, n = 13 Nrf2 KO), while the more severe “Fl-3@10” consisted of 45 K lux of light applied for 3 minutes starting 10 minutes after i.p. fluorescein injection (B, n = 11 B6J; D, n = 14 Nrf2 KO). OCT outer retinal thickness was then measured as the distance from the bottom of Bruch’s membrane to the external limiting membrane (BM_ELM) at day 5 and day 16 post light injury. Analysis shows recovery at day 16 after FCD-LIRD compared to day 5 measurement, both for B6J and Nrf2 KO mice. Each symbol represents mean values ± standard error of the mean for each day. * = P < 0.05, ** = P < 0.01, *** = P < 0.001.
Acknowledgments

Disclosure: B. Chen, None; B. Aredo, None; Y. Zhu, None; Y. Ding, None; C. Xin-Zhao, None; R.L. Ufret-Vincenty, None

References


**FIGURE 6.** Retinal functional recovery in Nrf2 KO mice after the fluorescein-assisted FCD-LIRD protocol. Nrf2 KO mice (n = 7) were treated with the moderate Fl-4@3 FCD-LIRD protocol (50 K lux of light applied for 4 minutes, starting 3 minutes after a 2 mg i.p. fluorescein injection). Scotopic Ganzfeld ERG a-wave and b-wave amplitudes were measured in response to low (0.1 log cd.s.m-2) and high (3.1 log cd.s.m-2) flash intensities at day 7 and day 14 post light injury. Compared to day 7 ERG readings, there was a significant functional recovery at day 14 post injury both in a-wave amplitude (A. 0.1 stimulus; C. 3.1 stimulus) and b-wave amplitude (B. 0.1 stimulus; D. 3.1 stimulus). Each symbol represents mean values ± standard error of the mean for each experimental group. * = P < 0.05, *** = P < 0.001.


