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Characterization of Multiple Light Damage Paradigms Reveals Regional Differences in Photoreceptor Loss

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Abstract

Zebrafish provide an attractive model to study the retinal response to photoreceptor apoptosis due to its remarkable ability to spontaneously regenerate retinal neurons following damage. There are currently two widely used light-induced retinal degeneration models to damage photoreceptors in the adult zebrafish. One model uses constant bright light, whereas the other uses a short exposure to extremely intense ultraviolet light. Although both models are currently used, it is unclear whether they differ in regard to the extent of photoreceptor damage or the subsequent regeneration response. Here we report a thorough analysis of the photoreceptor damage and subsequent proliferation response elicited by each individual treatment, as well as by the concomitant use of both treatments. We show a differential loss of rod and cone photoreceptors with each treatment. Additionally, we show that the extent of proliferation observed in the retina directly correlates with the severity of photoreceptor loss. We also demonstrate that both the ventral and posterior regions of the retina are partially protected from light damage. Finally, we show that combining a short ultraviolet exposure followed by a constant bright light treatment largely eliminates the neuroprotected regions, resulting in widespread loss of rod and cone photoreceptors and a robust regenerative response throughout the retina.

Keywords

NeuroD; transgene; zebrafish; retina; photoreceptor regeneration; light-damage; light induced retinal degeneration

1. Introduction

Photoreceptor apoptosis is the final pathway in many human retinal degenerative diseases, including age-related macular degeneration (AMD) and retinitis pigmentosa (RP) (Chang et al., 1993; Marc et al., 2008; Portera-Cailliau et al., 1994). Light-induced retinal degeneration (LIRD) is an established method to induce photoreceptor apoptosis and has many advantages over other models of AMD and RP. LIRD leads to faster photoreceptor

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degeneration than observed in genetic animal models of AMD and RP. In addition, LIRD initiates cell death of large numbers of photoreceptors in a synchronized manner, as opposed to genetic models, where photoreceptors are at various stages of health or degeneration (Wenzel et al., 2005). Further, LIRD selectively destroys photoreceptors, leaving the inner retina largely intact (Vihtelic and Hyde, 2000; Vihtelic et al., 2006). Finally, LIRD can be used in many different vertebrate species, including rats, mice, frogs, and fish, which allows researchers to take advantage the unique biological and practical strengths of each of these vertebrate model systems.

Adult zebrafish possess a unique ability to spontaneously regenerate retinal tissue following damage. Numerous damage paradigms have been established to induce retinal regeneration in the adult zebrafish, including the use of cytotoxins (Fimbel et al., 2007; Montgomery et al., 2010; Sherpa et al., 2008), laser ablation (Wu et al., 2001), physical perforation (Fausett and Goldman, 2006), and constant bright light treatment (Vihtelic and Hyde, 2000). While these techniques vary in selectivity and severity of injury, the cellular mechanism of regeneration is conserved in each case. Following neuronal cell death, increased proliferation is observed in two distinct locations. In the inner nuclear layer (INL), Müller glial cells re-enter the cell cycle to create large numbers of retinal progenitor cells that are capable of differentiating into any retinal cell type lost during injury (Fimbel et al., 2007; Otteson et al., 2001; Raymond et al., 2006; Thummel et al., 2008b; Vihtelic and Hyde, 2000). The second group of proliferating cells observed following retinal damage are termed rod precursors, which represent progenitor cells that are scattered throughout the outer nuclear layer (ONL) of the retina and differentiate only into rod photoreceptors. (Johns and Fernald, 1981; Otteson et al., 2001; Thummel et al., 2010). Although it is not clear what percentage of rod photoreceptors regenerate from rod precursors and what percentage regenerate from Müller glial-derived progenitors, Müller glial cells are considered the major source of retinal progenitors due to their robust proliferative response (Raymond et al., 2006). However, rod precursors were reported to regenerate normal numbers of rod photoreceptors in the absence of Müller glial cell proliferation (Thummel et al., 2010).

There are currently two LIRD paradigms used to damage photoreceptors in adult zebrafish. Constant exposure to bright light (~8,000 lux) is a widely used technique that causes photoreceptor loss in the dorsal half of the adult *albino* zebrafish retina within 24 hours of light onset (Vihtelic and Hyde, 2000; Vihtelic et al., 2006). This LIRD paradigm is based on the parameters previously shown to damage photoreceptors in the rodent (Noell et al., 1966; Wenzel et al., 2005). Adolescent *albino* mice show a peak of photoreceptor cell death at 36 hours after light onset (Wenzel et al., 2005), loss of photoreceptor inner and outer segments at 3 days (Yamashita et al., 1992), and severe outer nuclear layer (ONL) damage after 5 to 7 days of exposure (Yamashita et al., 1992). Both zebrafish and rodents exhibit significantly more photoreceptor damage in the superior half of the retina, which is analogous to the dorsal half of the adult zebrafish retina (Rapp and Williams, 1980). In addition, the constant bright light paradigm reportedly damages rod photoreceptors and leaves cone photoreceptors largely intact (Cicerone, 1976; La Vail, 1976; Thummel et al., 2010). More recently, a second LIRD paradigm has been used to cause photoreceptor apoptosis in adult zebrafish (Bernardos et al., 2007). This method uses a short exposure to an ultraviolet (UV) light source, which produces ~100,000 lux of light. 24 hours after a 30 min UV exposure, adult zebrafish show extensive damage to both rod and cone photoreceptors (Bernardos et al., 2007). However, this method reportedly causes the most damage to the central retina, surrounding the optic nerve, and a variable level of damage peripherally into the dorsal and ventral halves of the retina (Bernardos et al., 2007). Thus, this LIRD paradigm can damage both rod and cone photoreceptors, but results in areas with widespread photoreceptor loss adjacent to areas with minimal damage (Bernardos et al., 2007).

Recent advancements allow for the expression of a specific gene of interest to be knocked down during retinal regeneration (Fausett et al., 2008; Thummel et al., 2008b). This has revealed a small number of genes that are required for Müller glial cell proliferation and the subsequent amplification of Müller glia-derived retinal progenitors (Fausett et al., 2008; Ramachandran et al., 2010; Ramachandran et al., 2011; Thummel et al., 2010; Thummel et al., 2008a). It is possible that the genetic pathways that underlie these early steps of Müller glial cell activation and proliferation are conserved regardless of which retinal cell type is damaged. However, the later stages of progenitor cell migration and differentiation are inherently dependent on what cell type is being regenerated. For example, determining whether a particular gene of interest is required for rod vs. cone cell regeneration has proven difficult when using the constant bright light paradigm, because this model damages significantly more rods than cones (Qin et al., 2011; Thummel et al., 2010). Given the complex cellular events observed in regenerating specific retinal neurons, including proliferation, migration and differentiation, it is likely that different genetic pathways are required for the selective regeneration of rod and cone photoreceptors (Morris et al., 2011; Morris et al., 2008; Morris et al., 2005; Qin et al., 2011; Thummel et al., 2010). Thus, there is a need to establish a LIRD paradigm that achieves consistent damage to both rod and cone photoreceptors and widespread photoreceptor damage throughout the retina. Minimally, if data from these different LIRD paradigms can be interpreted, a clear analysis of the regional differences in photoreceptor loss that are observed in each of these models must be performed.

Here we present a thorough analysis of the two widely-used LIRD paradigms in adult zebrafish and show that each treatment results in differential damage to rod and cone photoreceptors. In addition, we show that the coincident use of both treatments results in significantly greater damage to rod and cone photoreceptors than each treatment used separately. Comparing the various light treatments, we provide clear evidence that the extent of photoreceptor loss directly correlates with the percentage of Müller glia that re-enter the cell cycle and the number of retinal progenitors that are formed. Finally, we show that zebrafish retinas contain two regions that are resistant to light damage, the previously-reported ventral retina, and a heretofore unreported posterior retina. Notably, combining the UV exposure with constant bright light treatment largely eliminates both of these protected areas, resulting in widespread damage to both rod and cone photoreceptors and a robust regenerative response throughout the retina.

2. Materials and methods

2.1 Zebrafish lines and maintenance

Three zebrafish lines were used in this study: *albino (alb)*, *Tg(nrd:EGFP)/alb* (Obholzer et al., 2008), and *Tg(gfap:EGFP)/alb* (Kassen et al., 2007). Fish were fed a combination of brine shrimp and dried flake food three times daily and maintained at 28.5°C on a 14 hour light (250 lux): 10 hour dark cycle (Westerfield, 1995). All animal care and experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee at Wayne State University School of Medicine and are in compliance with the ARVO statement on the use of animals in vision research.

2.2 Light treatment protocols

Following a 10 day dark adaptation, 6–9 month old zebrafish were exposed to one of three different light treatments to destroy photoreceptors: A four-day exposure to constant bright light (Kassen et al., 2007; Thummel et al., 2008a; Vihtelic and Hyde, 2000) using four halogen lamps (250 Watts; ~8,000 lux); and a 30-minute exposure to very intense light (~100,000 lux) using a UV light source, after which the animals were returned to standard 14

hour light: 10 hour dark conditions (Bernardos et al., 2007); a 30-minute exposure to the very intense UV light immediately followed by a four day exposure to constant bright light using the halogen lamps. We observed no change in water temperature during the 30-minute exposure to UV light. During the four-day exposure to constant bright light, the water temperature was maintained between 30–32°C. The physical setup of the constant bright light paradigm and the UV light paradigm are shown in Supplemental Figures 1 and 2, respectively.

2.3 Immunohistochemistry and confocal microscopy

Untreated control and light-damaged fish were euthanized at 36 and 48 hours after light onset and whole eyes were harvested. The tissue was fixed in 9:1 ethanolic formaldehyde (100% ethanol: 37% formaldehyde) overnight at 4°C, followed by cryoprotective washes in 5% sucrose/1X PBS at room temperature and 30% sucrose/1X PBS overnight at 4°C. Tissue was then incubated in a solution (1:1 ratio) of 30% sucrose/1X PBS: Tissue Freezing Medium (TFM, Triangle Biomedical Sciences, Durham, NC) overnight at 4°C, embedded in TFM and frozen at –80°C. Eyes were cryosectioned at 16 microns, transferred onto glass slides, dried at 55°C for 2 hours, and stored at –80°C.

Immunohistochemistry was performed as previously described (Thummel et al., 2008a). Slides were incubated overnight at room temperature using the following primary antibodies diluted in blocking solution: rabbit polyclonal anti-green fluorescent protein (GFP) antisera (1:1,500, Abcam, Cambridge, MA), mouse monoclonal anti-GFP antibody (1:200, Sigma Chemical, St. Louis, MO), mouse monoclonal anti-Proliferating Cell Nuclear Antigen (PCNA) antibody (1:1000, Sigma Chemical), rabbit polyclonal anti-PCNA antisera (1:100, AnaSpec, Fremont, CA), mouse monoclonal Zpr-3 and Zpr-1 antibodies (1:200, Zebrafish International Resource Center, Eugene, OR), rabbit polyclonal anti-blue opsin (1:500, (Vihtelic et al., 1999)) and rabbit polyclonal anti-UV opsin (1:1000, (Vihtelic et al., 1999)). Secondary antibodies included AlexaFluor goat anti-primary 488 and 594 (1:500, Invitrogen, Grand Island, NY). Coverslips were mounted using ProLong Gold (Molecular Probes, Eugene, OR).

Confocal microscopy was performed with a Leica TCS SP2 confocal microscope. Unless otherwise noted, images were obtained equidistant from the margin and the optic nerve in both the dorsal and ventral halves of the retina. For Figure 6, sections were collected from the anterior, central, and posterior retina and images were collected equidistant from the margin and midline in the dorsal half of the retina. For all other figures that utilized immunohistochemistry, sections containing the optic nerve were used.

Quantification of the number of EGFP-positive rod photoreceptor somas (Figure 1), the number of each cone photoreceptor cell type (Figure 2), and the percentage of PCNA-positive Müller glia (Figure 3), was performed in both the dorsal and ventral halves of the retina over a linear distance of 300 microns, equidistant from the margin and the optic nerve. Quantification of the number of each cone cell type was performed on 6 micron z-series-stacked images (0.5 micron per stack) obtained on a Leica TCS SP2 confocal microscope. Quantification of the number of EGFP-positive rod photoreceptor somas and the percentage of PCNA-positive Müller glia (Figure 3) were performed on images obtained on a Zeiss Axioplan 2 Imaging Apotome microscrope. A minimum of 5 retinas were used for each group. Statistical differences between groups ($p < 0.05$) was performed using student's t-test.

2.4 Western blot analysis

Western blot analysis was performed as previously described (Thummel et al., 2008b; Vihtelic et al., 1999). Total protein was isolated from whole embryos ($n=50-200$) at 48

hours post fertilization (hpf), undamaged control adult retinas (n=10) or light damaged adult retinas (n=10 per light treatment), and homogenized in extraction buffer (10% Glycerol, 1% Triton X-100, 5mM KPO₄, 0.05mM EDTA, 1 complete protease inhibitor cocktail tablet from Roche). The total protein equivalent of 1 embryo or 1/4 retina was combined with 2X Laemmli sample buffer (Bio-Rad, 161-0732), boiled for 4 min, electrophoresed through a 10% SDS-PAGE gel and then transferred to a PVDF membrane (Amersham Hybond-P, Piscataway, NJ). The membrane was blocked for 1 hour in 1X TTBS (40mM Tris-HCl, pH8.3, 0.5M NaCl, 0.2% Tween 20)/5% nonfat dry milk, followed by overnight incubation at 4°C in blocking buffer with one of the following primary antibodies: mouse monoclonal Zpr-1 antibody (1:500, Zebrafish International Resource Center), mouse monoclonal anti-PCNA antibody (1:500, Sigma Chemical), rabbit polyclonal anti-Cyclin H antisera (1:250, AnaSpec), rabbit polyclonal anti-Cdk-1 antisera (1:250, AnaSpec), rabbit polyclonal anti-Cdk-2 antisera (1:250, AnaSpec), or mouse monoclonal anti-β-actin antibody (1:20,000, Santa Cruz Biotechnology, Inc. Santa Cruz, CA). The blots were washed 3X for 15 minutes in 1X TTBS at room temperature, incubated with secondary antibody in blocking buffer for 1 hour at room temperature, then washed 3X for 15 minutes in 1X TTBS. Protein was detected with Western Lightning plus-ECL reagents (PerkinElmer, Inc). Experiments were performed in biological and technical triplicate and band density analyzed using AlphaVIEW SA software. Two bands were observed for Cdk-1 and Cdk-2, representing unphosphorylated (lower) and phosphorylated (upper) forms of each protein (Jamil et al., 2005; Watanabe et al., 1999). In each case, the lower band was used for band density analysis. Statistical analysis was performed using a student's T-test with a significant p value of <0.05.

2.5 Analysis of flat-mounted retinas

Retinas from adult *Tg(nrd:egfp)/alb* zebrafish were collected from either untreated control fish or light-damaged fish 48 hours after light onset. Eyes were enucleated using bent #5/45 forceps (WPI, Sarasota, FL) and placed cornea side down on a square #1.5 glass cover slip (VWR, Radnor, PA) under a light microscope. The optic stalks were removed using McPherson-Vannas curved micro-scissors (WPI). One prong of the scissors was inserted into the hole created by removal of the optic stalk and the sclera and retina were ventrally cut along the choroid fissure to the edge of the cornea. The eyes were positioned with the cornea side up and the retina and lens were then separated from the sclera through the ventral cut by gently applying pressure on the lens through the cornea with two pairs of #55 Dumont tweezers (WPI). The lens was then removed from the retinal tissue, which was gently maneuvered to lie flat with the photoreceptor side down on the glass cover slip. The cover slips were marked with a Sharpie permanent marker to demarcate the anterior side of the retinal flatmount. The cover slips were placed in 6-well polystyrene plates and the retinas were fixed in 100 00B51 of 4% paraformaldehyde at 4°C for 5–8 hrs, rehydrated in 1x PBS for 20 min, and then mounted onto Superfrost plus glass slides (VWR) using Prolong Gold antifade reagent (Invitrogen). Gross inspection and stereoscopic detection of EGFP was performed using a Leica M205 FA fluorescent stereoscope. Confocal imaging was performed using a Leica TCS SP2 microscope. The microscope stage was zeroed with the confocal plane within the INL, which was identified via the unique and invariant cellular EGFP expression pattern in bipolar cells. Detection of *nrd:EGFP* from rod inner segments was imaged from confocal planes +21–24 μm and rod outer segments were detected at confocal planes +40–43 μm.

ImageJ was used to analyze the fluorescent intensity of the EGFP-positive RIS, as previously described (Forrester/Berman 2011 and Sidi/Look 2008). Briefly, images from the anterior and posterior regions of flatmounted retinas (n=6–8) were imported into Image J as an “Image Sequence” and processed equally to subtract background and set threshold

values. Next, using the ROI manager and “Rectangular Sections” tool, 5 non-overlapping rectangles were drawn on each of the four corners and the center of the image and saved so that each image could be analyzed for fluorescent intensity within the same five areas. Finally, each image in the series was analyzed for the fluorescent intensity. Statistical differences between groups ($p < 0.05$) was determined by a student's t-test.

3. Results

3.1 Combining UV exposure with constant bright light results in extensive damage to rod and cone photoreceptors

To test whether various light treatments resulted in differences in rod photoreceptor damage, the *Tg(nrd:egfp)/alb* transgenic line was used to visualize rod photoreceptor cell somas in the outer nuclear layer (ONL), rod inner segments (RIS), and rod outer segments (ROS). Untreated (0 hr) fish showed EGFP-positive rod photoreceptors throughout the dorsal and ventral halves of the retina (Fig. 1A and C, respectively). At 48 hours of constant bright light (48 hLt), rod photoreceptor loss was evident in both the dorsal and ventral halves of the retina (Fig. 1B and D, respectively). As was previously reported in both rodents and zebrafish, the dorsal retina appeared to suffer more damage than the ventral retina (compare Fig 1B with Fig. 1D). Compared with 48 hLt retinas, UV exposure resulted in visibly fewer rod photoreceptors in both the dorsal and ventral halves of the retina 48 hours after light onset (48 hpUV) (Fig. 1E and G, respectively). EGFP-positive cellular debris was present in the degenerated ROS and RIS in the dorsal half of 48 hpUV retinas (Fig. 1E), whereas intact ROS and RIS were visible in the ventral half of 48 hpUV retinas (Fig. 1G). Combining UV exposure with 48 hours of constant bright light (UV+48hLt) resulted in the greatest amount of damage to both the dorsal and ventral halves of the retina (Fig. 1F and H, respectively). Very few rod photoreceptor somas and RIS were observed in the dorsal half of UV+48 hLt retinas, and the ROS was completely absent (Fig. 1F). Notably, extensive damage to the ROS and RIS was also visualized in the ventral half of UV+48 hLt retinas. (Fig. 1H).

Quantification of EGFP-positive rod photoreceptor somas in the ONL confirmed the results obtained by immunohistochemical analysis. Compared with untreated retinas, 48 hours of constant bright light (48 hLt) resulted in a significant reduction (48.5%) of rod photoreceptor somas in the dorsal half of the retina (Table 1, $p < 0.001$), but not the ventral half of the retina (Table 1, $p = 0.05$). Compared with 48 hLt retinas, 48 hpUV retinas showed significantly fewer rod photoreceptor somas in the dorsal half of the retina (Table 1, $p < 0.001$), but not the ventral half of the retina (Table 1, $p = 0.085$). Finally, compared with 48 hpUV retinas, UV+48 hLt retinas showed a similar amount of rod photoreceptor loss in the dorsal half of the retina (Table 1, $p = 0.14$), but significantly more loss in the ventral half of the retina (Table 1, $p < 0.001$).

Previous reports indicated that constant bright light resulted in more damage to rod photoreceptors than cone photoreceptors in adult zebrafish (Thummel et al., 2010; Thummel et al., 2008a). To determine whether the various light treatments resulted in significant differences in the loss of cone photoreceptors, untreated and light-damaged retinas were immunolabeled with cone-cell specific markers (Vihtelic et al., 1999). UV opsin was used to label short single cones (uv cones), Zpr-1 was used to label red/green double cones, and blue opsin was used to label long single cones (blue cones). The dorsal half of untreated retinas contained an average of 45.6 ± 2.3 uv cones, 94.2 ± 8.0 double cones, and 48.4 ± 0.9 blue cones per 300 micron linear length, whereas the ventral half of untreated retinas contained an average of 56.4 ± 5.6 uv cones, 104.6 ± 4.3 double cones, and 60.4 ± 3.4 blue cones per 300 micron linear length (Table 1). Following 48 hours of constant bright light (48 hLt), all the cone types were reduced in number and appeared shortened and hypertrophied (Fig. 2B, F, J, N, R, V, Y, Z) relative to the undamaged controls. However, a significant loss was only

observed in double cones and blue cones in the ventral retina (Table 1, $p<0.001$ and $p=0.02$, respectively). Compared with untreated retinas, UV exposure resulted in significantly fewer uv cones (21.8 ± 8.1), double cones (21.3 ± 6.1), and blue cones (30.3 ± 3.0) in the dorsal retina (Fig. 2C, K, S, Y; Table 1, $p=0.02$, $p<0.001$, $p<0.001$, respectively), but not in the ventral retina (Fig. 2G, O, W, Z). In contrast, the UV+48 hLt retinas showed significant loss of all three cone cell types in both the dorsal and ventral retinas (Fig. 2D, H, L, P, T, X, Y and Z, Table 1) relative to untreated control retinas. In addition, compared with 48 hpUV retinas, UV+48 hLt retinas showed significantly fewer blue cones in the dorsal and ventral retinas (Fig. 1Y and Z, Table 1, $p<0.001$), and significantly fewer uv cones in the ventral retina (Fig. 1Z, Table 1, $p=0.01$). Finally, in the ventral half of 48 hpUV and UV+48 hLt retinas, the damage appeared to abruptly stop near the ventral-most portion of the retina (Fig. 2G, H, and P), indicating a strong protection of this retinal region.

Taken together, these data suggest that combining UV exposure with constant bright light resulted in significantly more rod and cone photoreceptor loss than any single light treatment alone. Although significant differences were observed in both dorsal and ventral halves of the retina, UV+48 hLt retinas showed the most damage in the ventral half of the retina relative to the other light treatments.

3.2 The regeneration response of the retina correlates with the extent of photoreceptor damage

Previous reports indicated that Müller glial cells re-entered the cell cycle 31–36 hours after constant bright light onset (Kassen et al., 2007; Vihtelic and Hyde, 2000). The *Tg(gfap:egfp)/alb* transgenic line was used to visualize EGFP-positive Müller glial (Thummel et al., 2008b). To visualize Müller glial proliferation during the various light treatments, the *Tg(gfap:egfp)/alb* retinas were co-labeled with Proliferating Cell Nuclear Antigen (PCNA), an established marker for the G1/S transition (Thummel et al., 2006; Thummel et al., 2010; Thummel et al., 2008a; Thummel et al., 2004). In undamaged retinas (0 hr), EGFP-positive Müller glial were observed throughout the dorsal and ventral halves of the retina, with their cell bodies located in the inner nuclear layer (INL), and their processes extending from the nerve fiber layer to the outer limiting membrane (Fig. 3A and C). PCNA-positive rod precursors were commonly observed in the ONL, contributing to the persistent neurogenesis of rod photoreceptors in adult zebrafish (Fig. 3A, arrow). However, PCNA-positive Müller glial cells were very rarely observed (Table 2). Following 36 hours of constant bright light (36 hLt), the percentage of PCNA-positive Müller glia in the dorsal and ventral halves of the retina was $15.2\pm 6.3\%$ and $3.8\pm 3.4\%$, respectively (Fig. 3B, D and I, Table 2). Consistent with a higher level of photoreceptor loss, 36 hpUV retinas exhibited an even higher percentage of PCNA-positive Müller glia, $28.7\pm 3.1\%$ in the dorsal half of the retina and $25.9\pm 2.8\%$ in the ventral half of the retina (Fig. 3E, G and I, Table 2). However, compared with the other treatments, UV+36 hLt retinas contained a significantly higher percentage of PCNA-positive Müller glia in both the dorsal and ventral halves of the retinas ($56.7\pm 3.5\%$ and $53.4\pm 8.5\%$, respectively, Fig. 3F, H and I, Table 2). Thus, the percentage of Müller glia that re-enter the cell cycle 36 hours after light onset correlates with the extent of photoreceptor cell loss.

Müller glial cell division at 36 hours after light onset produces clusters of PCNA-positive progenitors within 12 hours (Kassen et al., 2007; Vihtelic and Hyde, 2000), at which point these progenitors begin to migrate to the ONL to replace the lost photoreceptors. Similar to the findings at 36 hours after light onset, we observed the greatest proliferation response in UV+48 hLt retinas (Fig. 4D and H). This was especially apparent when comparing the dorsal and ventral halves of the retina (compare Fig. 4E–H). Compared with the other light treatments, UV+48 hLt retinas showed the highest amount of PCNA-positive clusters in the ventral half of the retina (Fig. 4H).

To quantify the regeneration response at 48 hours after light onset, analysis of band intensity on immunoblots was performed from protein collected from 48 hLt, 48 hpUV, and UV+48 hLt retinas. Protein from 48 hour post fertilization (hpf) embryos and untreated retinas (0 hr) were used for controls. Significant reduction in Zpr-1 expression was observed between the 0 hour and 48 hour timepoints under all three light treatments (Fig. 5A and B, Table 3), representing the loss of double cone photoreceptors. These data are consistent with Zpr-1 immunolocalization on light-treated retinas, in which UV+48 hLt retinas exhibited the fewest numbers of double cone photoreceptors of all three light treatments (Fig. 2Y and Z, Table 2). Immunoblot analysis also revealed significant differences in cell cycle markers between the various light treatments (Fig. 5A, Table 3). PCNA expression levels significantly increased from 0 hr→48 hLt→48 hpUV→UV+48 hLt (Fig. 5A and B, Table 3), which is consistent with PCNA immunolocalization in these retinas (Fig. 4). Expression of Cyclin H and two Cyclin-dependent kinases, Cdk-1 and Cdk-2, were also analyzed. Cyclin H binds Cdk-7 to form a Cdk-Activating Kinase (CAK) (Fesquet et al., 1993). CAK regulates cell cycle progression by phosphorylating, and thus activating, certain cyclin-dependent protein kinases, including Cdk-1 and Cdk-2 (Devault et al., 1992; Fesquet et al., 1993; Gu et al., 1992). Each light treatment resulted in increased Cyclin H expression (Fig. 5A, Table 3). Two bands were observed for Cdk-1 and Cdk-2, representing different states of phosphorylation for each protein (Jamil et al., 2005; Watanabe et al., 1999). Phosphorylation of Cdk-1 on Thr-160 by CAK and is necessary to activate the Cdk-1/Cyclin B complex (Devault et al., 1992; Fesquet et al., 1993). Dephosphorylation of Thr-14 and Tyr-15 by Cdc25 completes the activation of the complex and initiates the transition from prophase into metaphase (Devault et al., 1992). Both the upper and lower Cdk-1 bands increased in light-treated retinas (Fig. 5A), indicating that both inactive and active forms of the protein increased during the light treatment. Similar to Cdk-1, full activation of Cdk-2 requires dephosphorylation of Thr-14 and Tyr-15 by Cdc25 and phosphorylation of Thr-160 by CAK (Gu et al., 1992). Cdk-2 binds with Cyclin E during the G1/S transition and with Cyclin A during S phase (Morgan, 1997). For Cdk-2 expression, light treatment resulted in a decrease of the upper band and an increase in the lower band (Fig. 5A). Compared with the other light treatments, UV+48 hLt retinas showed the highest amount of PCNA, Cyclin H, Cdk-1, and Cdk-2 (Fig. 5A, Table 3).

3.3 PCNA immunolocalization and analysis of flatmounted retinas reveal regional differences in photoreceptor damage following light exposure

Previous data indicate a strong protection of the ventral half of the retina to light damage (Vihtelic et al., 2006). Combining UV exposure and constant bright light resulted in the most significant damage to the ventral half of the retina, however, differences between the dorsal and ventral halves of the retina were still observed (Figs. 1 & 2). To determine whether a similar protective mechanism was present in anterior or posterior halves of the retina, untreated and 48 hour light-damaged eyes were sectioned in an anterior-to-posterior manner along the dorsal ventral axis and analyzed for PCNA immunolocalization. No differences were observed in undamaged (0 hr) retinas (Fig. 6A, E, I, and M). However, in each of the light treatment paradigms, more PCNA immunolocalization was observed in the anterior retinal sections than the posterior retinal sections (Fig. 6), indicating a protection of the posterior retina to light damage. In addition, as was the case with protection of the ventral half of the retina, combining UV and constant bright light largely eliminated the protection of the posterior region of the retina (Fig. D, H, L, and P).

To better visualize and quantify this protected region, undamaged and light-treated *Tg(nrd:egfp)/alb* retinas were flat-mounted to clearly visualize the EGFP-positive rod photoreceptors in the anterior and posterior halves of the retina (Fig. 7, 8). Confocal microscopy was used to determine the various layers of the retina, including the INL, RIS,

and ROS (Fig. 7C–E, respectively). Since ROS were largely absent in all light-treated retinas (Fig. 7H), confocal microscopy of RIS was used to best discriminate the extent of rod photoreceptor damage in the anterior and posterior halves of the retina following the various light treatment paradigms. In untreated (0 hr) retinas, RIS formed a distinct honeycomb pattern that surrounded the cone photoreceptor somas (Fig. 8A). In 48 hLt retinas, a significant reduction in RIS was observed in both the anterior and posterior retinas (Fig. 8B, F, I, Table 4). However, significantly more damage occurred in the anterior retina when compared with the posterior ($p < 0.001$). 48 hpUV retinas also showed significant loss of RIS compared with untreated retinas ($p < 0.001$), but exhibited no differences between the anterior and posterior retinas (Fig. 8C, G, I, Table 4, $p = 0.99$). Finally, compared with the other light treatments, UV+48 hLt retinas showed the most significant loss of RIS (Fig. 8D, H, I, Table 4). In addition, similar to 48 hLt retinas, the UV+48 hLt retinas also exhibited significantly more damage in the anterior retina (Fig. 8I, Table 4, $p = 0.002$). Taken together, these data indicate that both the posterior and ventral portions of the retina are partially protected from light damage. In addition, concomitant use of UV exposure and constant bright light largely eliminates this protection, allowing for widespread damage of both rod and cone photoreceptors and a robust proliferation response throughout the retina.

4. Discussion

4.1 Summary of findings

There are currently two widely-used models to induce photoreceptor apoptosis in adult zebrafish: constant bright light of ~8,000 lux and a short exposure to ~100,000 lux of a UV light source. Here we report a thorough analysis of the photoreceptor damage and the subsequent proliferation response elicited by each individual treatment, as well as by the concomitant use of both treatments. We show that constant bright light primarily damages rod photoreceptors, leaving the cone photoreceptors largely intact (Figs. 1 and 2, Table 1). In contrast, we show that UV treatment results in significant damage to both rods and cones (Figs. 1 and 2, Table 1). Each of these treatments results in more damage to the dorsal retina than to the ventral retina (Figs. 1 and 2, Table 1). Combining the UV exposure followed by constant bright light results in significantly greater loss of both rods and cones to the dorsal and ventral retinas, largely eliminating the neuroprotection of the ventral retina (Figs. 1 and 2, Table 1). Additionally, we show that the extent of photoreceptor damage with each treatment directly correlates with the percentage of Müller glia that re-enter the cell cycle and the subsequent amplification of retinal progenitors (Figs. 3 and 4, Table 2). Finally, we provide strong evidence for a previously-undiscovered protection of the posterior retina to light damage and conclude that both the ventral and posterior portions of the retina are naturally resistant to light lesion (Figs. 6–8).

4.2 Practical considerations

In addition to the scientific considerations already detailed, there are many practical considerations to consider with the use of each of these light damage paradigms. A constant bright light setup can be acquired for under \$100 with materials from local hardware and pet supply stores. It consists of four 250W halogen lights, a thermometer, a small fan (to keep the water temperature from getting too hot), and a water aerator (Supp. Fig. 1). Although this setup is relatively inexpensive, it takes up approximately 12 square feet of bench space and only ~20–24 fish can be treated at a time. In addition, this setup cannot be placed in the fish facility, because the constant lights would disrupt the circadian rhythm of the other fish. In contrast, the UV treatment setup (Supp. Fig. 2) is relatively expensive, as it requires a UV source. However, due to the short exposure (30 minutes), multiple groups can be treated in succession. We place ~10 fish in a 250 mL beaker that is partially covered in aluminum foil (to reflect the UV light back into the beaker). The small beaker is placed inside a larger 4L

beaker filled with ~ 500 mL of water. The optic cable from the UV source is secured outside the large beaker pointing at the fish and a cardboard box is placed around the entire apparatus before turning on the UV light. Thus, although the UV treatment is faster and the setup is smaller, there is a significant cost to consider in purchasing a UV source, which may not be available to every research laboratory.

4.3 Variable response of Müller glial cell proliferation in light-damage paradigms

Many human retinal degenerative diseases, such as AMD and RP, result in photoreceptor apoptosis (Chang et al., 1993; Marc et al., 2008; Portera-Cailliau et al., 1994). As a result, underlying Müller glial cell hypertrophy and undergo reactive gliosis. In damaged zebrafish retinas, Müller glial cells also hypertrophy and undergo a gliosis-like response (Fig. 3) (Thummel et al., 2008a). However, in zebrafish, this leads to Müller glial cell proliferation and a generation of large numbers of retinal progenitors (Figs. 3 and 4), whereas in the damaged mammalian retina, Müller glia upregulate fibrous proteins and form a glial scar (Jones and Marc, 2005). The extent of reactive gliosis and glial scar formation in the mammalian retina correlates with the extent of photoreceptor damage (Marc et al., 2008). We show that Müller glial cells in the zebrafish retina are also sensitive to the level of photoreceptor damage (Figs. 3 and 4, Table 2). When the photoreceptor damage is relatively minor, such as with the constant bright light treatment, a small percentage of Müller glial cells re-enter the cell cycle and produce a modest number of progenitors (Fig. 3, Table 2). In contrast, when UV exposure is combined with constant light treatment to damage large numbers of rods and cones, over half of the resident Müller glial cells re-enter the cell cycle and a large number of progenitors are produced (Figs. 3 and 4, Table 2). These data imply that in cases of even greater damage to the retina, such as combining a light-lesion model with a cytotoxin model, an even greater number of Müller glial cells may re-enter the cell cycle. Alternatively, it is also possible that only a subset of Müller glial cells can respond and re-enter the cell cycle regardless of the extent of retinal damage. Recent evidence suggests that Müller glial cells in the injured mammalian retina can be induced to re-enter the cell cycle and produce small numbers of progenitors (Joly et al., 2011; Wan et al., 2008), and thus, may have the capacity to participate in a regenerative response. However, it is unclear how Müller glial cells interpret the extent of photoreceptor damage and how this in turn leads to either a healthy regeneration response or reactive gliosis. Comparing the underlying genetics of the variable responses of Müller glial cell proliferation observed in these damage paradigms could provide insight into the signaling mechanisms that control the extent of Müller glia cell reactivity.

4.4 Regional differences

Here we show that both the ventral and posterior regions of the retina are protected from light damage. It was previously reported that the dorsal (or superior) retina in adult *albino* zebrafish is more susceptible to light damage than the ventral (inferior) retina (Vihtelic et al., 2006). This is consistent with reports in the rodent, which show more severe damage to the superior half of the retina (Rapp and Williams, 1980). Many possibilities could account for this finding, with the main hypothesis being that Rhodopsin is the trigger for photoreceptor cell death (Grimm et al., 2000; Humphries et al., 1997; Noell et al., 1966). Thus, reduced photoreceptor damage in the ventral/inferior retina was attributed to shorter ROS, and thus, lower levels of Rhodopsin. Similar to the rodent, zebrafish also have shorter ROS in the inferior/ventral half of the retina (Fig. 1, Vihtelic et al., 2006), which may also account for the dorsal/ventral differences observed in light-damaged zebrafish retinas (Figs. 1 and 2, Vihtelic et al., 2006). However, there are no reports indicating that the posterior half of the zebrafish retina possesses fewer rod photoreceptors or shorter ROS. Further, analysis of flatmounted retinas (Fig. 6–8) indicated similar amounts of EGFP-positive RIS in untreated retinas (Fig. 8), suggesting that differences in Rhodopsin levels likely does not

account for the anterior/posterior differences observed in light-treated retinas. Another possibility for the regional differences is differential expression of neuroprotective factors, such as FGF-2 (Li et al., 2003; Liu et al., 1998; Stone et al., 1999). FGF-2 expression was reportedly higher in the inferior half of the rodent retina compared with the superior half (Stone et al., 1999). Given that FGF-2 protects photoreceptors from light damage in both rodents (Faktorovich et al., 1990, 1992; LaVail et al., 1991) and zebrafish (Qin et al., 2011), this may contribute to the regional differences observed in light-treated retinas. Although it is not known whether FGF-2 is differentially expressed in the adult zebrafish retina, FGF-2 was recently reported to regulate rod photoreceptor cell maintenance in adult zebrafish (Qin et al., 2011). A final possibility for the regional differences observed in light susceptibility is that photoreceptors in the both halves of the retina develop differently due to the animals being reared in environments with overhead lighting. When rodents are raised in animal facilities with room lighting at the side of the animals, rather than above the animals, the protection of the inferior retina was lost (Stone et al., 1999). Although it is unclear whether this is also the case in adult zebrafish, this would not account for the anterior/posterior differences observed in light-treated zebrafish retinas, since they are at the same level relative to the overhead lights. Therefore, it is currently unclear what accounts for the heretofore unrecognized protection of the posterior zebrafish retina.

Highlights

- Various light damage paradigms result in differences in photoreceptor loss
- Müller glial cell proliferation correlates with the severity of photoreceptor loss
- The ventral and posterior retina of adult zebrafish are protected from light damage

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Rod photoreceptor loss following various light damage paradigms

EGFP-positive rod photoreceptors in the dorsal (A, B, E, F) and ventral (C, D, G, H) halves of the retina from adult *Tg(nrd:egfp)/alb* zebrafish. A. Untreated (0 hr) dorsal retina with EGFP-positive somas in the outer nuclear layer (ONL), rod inner segments (RIS), and rod outer segments (ROS). B. Dorsal retina following a 48 hour exposure to constant bright light (48 hLt), with fewer EGFP-positive somas in the ONL, and truncated RIS and ROS. C. 0 hr ventral retina with undamaged ONL, RIS, and ROS. D. 48 hLt ventral retina, with fewer ONL nuclei, but intact RIS and ROS. E. Dorsal retina 48 hours after a 30 minute exposure to UV light (48 hpUV), with degenerated ROS, RIS, and ONL. F. Dorsal retina after a 30 minute exposure to UV light followed by 48 hours of constant bright light (UV+48 hLt),

with degenerated ROS, RIS, and ONL. G. 48 hpUV ventral retina with fewer ONL and truncated RIS and ROS. H. UV+48 hLt ventral retina with degenerating ROS, RIS, and ONL. I. Quantification of the average number of rod photoreceptors per 300 microns in the dorsal and ventral halves of the retina following various light treatments. Significant differences between groups ($p < 0.05$) is indicated as: “a” for compared with Untreated (0 hr); “b” for compared with 48 hLt; and “c” for compared with 48 hpUV. Scale bar in panel A represents 75 microns.

Figure 2. Cone photoreceptor loss following various light damage paradigms

A – H. Short single cones (uv cones) are immunolabeled with UV opsin. I – P. Red/Green double cones are immunolabeled with Zpr-1. Q – X. Long single cones (blue cones) are immunolabeled with blue opsin. Untreated (0 hr) retinas show undamaged cones in both the dorsal and ventral halves of the retina (A, B, E, F, I, J, M, N, Q, R, U, V). Following 48 hours of constant bright light (48 hLt), uv and double cones appear short and hypertrophied (B, F, J, N), but are still visible in both the dorsal and ventral halves of the retina. 48 hours after a 30 minute UV exposure (48 hpUV), degeneration of all three cone types is evident in throughout the dorsal half of the retina (C, K, S), but not throughout the ventral half of the retina (G, O, W). After a 30 minute exposure to UV light followed by 48 hours of constant bright light (UV+48 hLt), only cone cell debris is evident in the dorsal half of the retina (D, L, T), and degeneration of all cone cell types is evident in the ventral half of the retina (H, P, X). Y–Z. Quantification of the average number of each cone type per 300 microns in the dorsal and ventral halves of the retina following various light treatments. Significant differences between groups ($p < 0.05$) is indicated as: “a” for compared with Untreated (0 hr); “b” for compared with 48 hLt; and “c” for compared with 48 hpUV. Scale bar in panel A represents 50 microns.

Figure 3. Müller glial cell proliferation in various light treatments

EGFP-positive Müller glial cells in the dorsal (A, B, E, F) and ventral (C, D, G, H) halves of the retina from adult *Tg(gfap:egfp)/alb* zebrafish (Green) co-labeled with PCNA (Red). A. Untreated (0 hr) dorsal retina, showing EGFP-positive Müller glial cells with somas located in the INL and a processes extending to beyond the ONL and GCL. Müller glial cells in 0 hr retinas are not PCNA-positive. B. A subset of Müller glial cells are PCNA-positive in the dorsal retina following 36 hours of constant bright light treatment (36 hLt). C. 0 hr ventral retina, showing PCNA-negative Müller glial cells. D. 36 hLt dorsal retina, showing PCNA-negative Müller glial cells. E. 48 hours after a 30 minute UV exposure (36 hpUV), a subset of Müller glial cells are PCNA-positive in the dorsal retina. F. After a 30 minute exposure to

UV light followed by 48 hours of constant bright light (UV+48 hLt), the majority of Müller glial cells are PCNA-positive in the dorsal retina. G. 36 hpUV retina, showing PCNA-positive Müller glial cells in the dorsal half of the ventral retina. H. UV+48 hLt retina, showing the majority of Müller glial cells are PCNA-positive in the ventral retina. I. Quantification of the percentage of PCNA-positive Müller glial cells in the entire dorsal and ventral halves of the retina in untreated and light-treated retinas. Significant differences between groups ($p < 0.05$) is indicated as: “a” for compared with Untreated (0 hr); “b” for compared with 36 hLt; and “c” for compared with 36 hpUV. Scale bar in panel A represents 50 microns.

Figure 4. PCNA immunolocalization following various light damage paradigms

A. Untreated (0 hr) dorsal retina, showing the absence of PCNA immunolocalization in the inner nuclear layer (INL). B – D. PCNA immunolocalization in the INL and ONL of the dorsal retina, following each of the light damage paradigms (48 hLt, 48 hpUV, and UV+48 hLt). E. 0 hr ventral retina, showing PCNA immunolocalization in the ONL, but not in the INL. F – G. Following 48 hLt (F) and 48 hpUV (G), isolated PCNA-positive cells are observed in the ONL and INL of the ventral half of the retina, however, the proliferation response is markedly less than that observed in the corresponding dorsal half of the retina. H. Ventral retinas from UV+48 hLt possess large numbers of PCNA-positive cells in the INL and ONL. Scale bar in panel A represents 50 microns.

Figure 5. Western blot analysis following various light damage paradigms

A. Expression levels of Zpr-1, PCNA, Cyclin H, Cdk-1, Cdk-2, and β -actin were determined in 48 hour post-fertilization embryos (48 hpf), untreated adult retinas (0 hr), and following the various light damage paradigms (48 hLt, 48 hpUV, and UV+48 hLt). Zpr-1 was not detected at 48 hpf since double cones are not present at this early stage in development. Zpr-1 expression decreased from 0 hr→48 hLt→48 hpUV→UV+48 hLt, representing a progressive loss of double cones. Conversely, PCNA and Cyclin H levels increased from 0 hr→48 hLt→48 hpUV→UV+48 hLt. Two bands were observed for Cdk-1 and Cdk-2, most likely representing phosphorylated and unphosphorylated forms of each protein. For Cdk-1, the upper band was not detected at 0 hr, and both the upper and lower bands increased from

0 hr→48 hLt→48 hpUV→UV+48 hLt. For Cdk-2, only the upper band was detected at 0 hr (and slightly at 48 hLt), and the lower band increased from 0 hr→48 hLt→48 hpUV→UV+48 hLt. B. Graphic representation of the average $\log_{10}$ band density of Zpr-1 and PCNA expression. Significant differences between groups ($p < 0.05$) is indicated as: “a” for compared with 48 hpf; “b” for compared with untreated (0 hr); “c” for compared with 48 hLt’ and “d” for compared with 48 hpUV.

Figure 6. Anterior/Posterior differences in light-induced proliferation response

Cryosections of the dorsal half of the retina, taken from the anterior (A–D), central (E–L), or posterior regions (M–P) of the retina were immunolabeled with PCNA (red). In untreated (0 hr) retinas (A, E, I, M), PCNA-positive cells were occasionally observed as rod precursors in the outer nuclear layer (ONL). Following 48 hours of constant bright light (48 hLt; panels B, F, J, and N), PCNA-positive cells were observed in the inner nuclear layer (INL) and ONL in sections from the anterior and central retina, but not the posterior retina. In addition, the proliferation response was much more robust in the anterior-most section (B). 48 hours after a 30 minute UV exposure (48 hpUV; panels C, G, K, and O), clusters of PCNA-positive cells were observed in the INL and ONL in sections from the anterior and central retina. The posterior retina (O), in contrast, possessed isolated PCNA-positive cells in both the INL and ONL, indicative of a more subdued proliferative response. After a 30 minute exposure to UV light followed by 48 hours of constant bright light (UV+48 hLt; panels D, H, L, and P), large clusters of PCNA-positive nuclei were present throughout the anterior, central, and posterior retina. Scale bar in panel A is 50 microns.

Figure 7. Orientation and imaging of flatmounted retinas

A. Schematic representation of the orientation used for these studies on flatmounted retinas. Anterior is to the left and posterior is to the right. B. Stereofluorescent image of an untreated, flatmounted *Tg(nrd:egfp)/alb* retina, oriented as shown in panel A. C – H. Confocal images taken at various focal planes in the anterior retina (equidistant between the dorsal and ventral halves) revealed the inner retina (INL), rod inner segments (RIS), and rod outer segments (ROS). C. EGFP-positive bipolar cells were detected in the INL of untreated retinas. D. EGFP-positive RIS exhibited the characteristic honeycomb pattern in untreated retinas. E. EGFP-positive ROS were undamaged in untreated retinas. F. Following 48 hours of constant bright light (48 hLt), EGFP-positive bipolar cells were present in the INL and

appeared hypertrophied. G. EGFP-positive RIS in 48 hLt retinas were damaged (note large EGFP-negative areas) and disorganized. H. ROS were not detected in 48 hLt retinas. Scale bar in panel B represents 450 microns. The scale bar in panel C (C–H) represents 100 microns.

Figure 8. Confocal microscopy of rod inner segments in flatmounted retinas reveal anterior/posterior differences in response to various light-damage paradigms
 Confocal images of EGFP-positive rod inner segments (RIS) were obtained from the central anterior (A–D) and central posterior (E–H) portion of flatmounted *Tg(nrd:egfp)/alb* retinas. Untreated (0 hr) retinas (A, E) showed the characteristic honeycomb pattern of RIS (note the inset in panel A) in both the anterior and posterior halves of the retina. Following 48 hours of constant bright light (48 hLt; panels B and F), the RIS in the anterior half of the retina were severely disrupted (B), but remained largely intact in the posterior half of the retina (F). 48 hours after a 30 minute UV exposure (48 hpUV; panels C and G), RIS in both the anterior and posterior halves of the retina were severely damaged. After a 30 minute exposure to UV light followed by 48 hours of constant bright light (UV+48 hLt; panels D and H), RIS in both the anterior and posterior halves of the retina were severely damaged. Compared with the other treatments, the anterior half of the UV+48 hLt retinas appeared to

sustain the greatest damage to RIS (compare panel D with all other panels). I. Quantification of the fluorescent intensity of EGFP-positive RIS relative to RIS in the posterior portion of the retina. Significant differences between groups ($p < 0.05$) is indicated as: “a” for significantly less than the untreated retina; “b” for significantly less than the posterior half of the corresponding treatment group; and “c” for significantly less than all other points. Scale bar in panel A represents 100 microns.

Table 1

Quantification of the average number of rod and cone photoreceptors per 300 microns following various light treatments.

Ave # of Photoreceptors per 300 microns ± SEM				P-Value			
	0 hr	48 hLt	48 hpUV	UV+48 hLt	0 hr vs 48 hLt	0 hr vs 48 hpUV	48 hLt vs 48 hpUV
Rods, Dorsal	81.4±1.9	41.8±2.0	9.4±1.7	5.6±1.6	4.86E-07	2.70E-09	1.84E-06
Rods, Ventral	78.6±2.7	67.8±3.8	59.8±1.4	27.2±2.1	0.0491	0.0002	0.0851
UV cones, Dorsal	45.6±2.3	45.4±3.6	21.8±0.0	5.6±2.1	0.9639	2.19E-02	0.0282
UV cones, Ventral	56.4±5.6	53.6±5.9	45.0±0.2	23.4±5.0	0.7383	1.62E-01	0.2921
Dbl cones, Dorsal	94.2±8.0	67.6±14.4	31.3±0.0	10.8±3.2	1.45E-01	2.22E-04	0.6094
Dbl cones, Ventral	104.6±4.3	73.8±4.8	60.3±0.6	36.8±15.2	2.01E-03	5.25E-02	0.7634
Blue cones, Dorsal	48.4±0.9	36.2±9.5	30.3±0.6	3.8±1.5	2.38E-01	3.40E-04	0.0308
Blue cones, Ventral	60.4±3.4	43.4±5.1	46.0±0.8	6±3.1	2.48E-02	8.20E-02	0.5529
							6.69E-04

Average percentage of Müller glial proliferation in dorsal and ventral halves of the retina following various light treatments.

Table 2

	Ave % of PCNA + Müller glia ± SEM				P-Value	36 hLt vs 36 hpUV	36 hLt vs 36 hpUV UV+36 hLt
	0 hr	36 hLt	36 hpUV	UV+36 hLt			
Dorsal	0.54±0.4	15.2±6.8	28.7±3.1	56.7±3.5	0.0496	1.98E-05	2.64E-07
Ventral	0.15±0.1	3.8±3.4	25.9±2.8	53.4±8.5	0.3173	1.62E-05	2.32E-04
							0.0003
							0.0149

Table 3
Quantification of the band intensity of Zpr-1, PCNA, Cyclin H, Cdk-1, and Cdk-2 expression on immunoblots.

	Ave log ₁₀ of Band Intensity ± SEM					P-Value	48 hLt vs 48 hpUV	48 hpUV vs UV+48 hLt
	48 hpf	0 hr	48 hLt	48 hpUV	UV+48 hLt			
Zpr-1	0.78±0.01	1.02±0.00	0.98±0.01	0.92±0.01	0.82±0.01	0.0018	1.24E-07	6.15E-14
PCNA	1.12±0.02	0.75±0.03	0.95±0.02	1.01±0.01	1.06±0.01	1.74E-04	5.51E-07	0.0123
Cyclin H	0.95±0.03	0.81±0.02	0.83±0.02	0.82±0.02	0.86±0.01	0.4185	0.7796	0.6225
Cdk-1	0.94±0.02	0.73±0.01	0.77±0.00	0.81±0.01	0.88±0.01	2.92E-05	3.08E-06	2.26E-09
Cdk-2	0.95±0.01	0.74±0.03	0.77±0.03	0.86±0.01	0.86±0.01	0.3703	0.0222	0.0005
								0.2151
								0.1037
								9.14E-05
								0.0112

Quantification of the fluorescent intensity of EGFP-positive rod inner segment (RIS) in the anterior and posterior halves of flatmounted Tg(*nrd:egfp*)/*alb* retinas.

Table 4

	% of Fluorescent Intensity ± SEM				P-Value	48 hL1 vs 48 hpUV	48 hpUV vs UV+48 hL1
	0 hr	48 hL1	48 hpUV	UV+48 hL1			
Anterior	96±5	22±8	26±13	10±15	6.12E-29	9.44E-22	7.13E-39
Posterior	100±6	48±6	26±14	22±15	6.77E-12	1.26E-16	7.8734E-05
P-Value of Ant vs Post	0.5802	3.97E-11	0.9877	0.0018			0.2279