Video Article

A Novel Light Damage Paradigm for Use in Retinal Regeneration Studies in Adult Zebrafish

Jennifer L. Thomas[1], Ryan Thummel[1,2]

[1]Department of Anatomy and Cell Biology, Wayne State University School of Medicine

[2]Department of Opthalmology, Wayne State University School of Medicine

Correspondence to: Ryan Thummel at rthummel@med.wayne.edu

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Abstract

Light-induced retinal degeneration (LIRD) is commonly used in both rodents and zebrafish to damage rod and cone photoreceptors. In adult zebrafish, photoreceptor degeneration triggers Müller glial cells to re-enter the cell cycle and produce transient-amplifying progenitors. These progenitors continue to proliferate as they migrate to the damaged area, where they ultimately give rise to new photoreceptors. Currently, there are two widely-used LIRD paradigms, each of which results in varying degrees of photoreceptor loss and corresponding differences in the regeneration response. As more genetic and pharmacological tools are available to test the role of individual genes of interest during regeneration, there is a need to develop a robust LIRD paradigm. Here we describe a LIRD protocol that results in widespread and consistent loss of both rod and cone photoreceptors in which we have combined the use of two previously established LIRD techniques. Furthermore, this protocol can be extended for use in pigmented animals, which eliminates the need to maintain transgenic lines of interest on the albino background for LIRD studies.

Video Link

The video component of this article can be found at http://www.jove.com/video/51017/

Introduction

Light-induced retinal degeneration (LIRD) is commonly used in both rodents and zebrafish to damage rod and cone photoreceptors. In adult zebrafish, photoreceptor degeneration triggers Müller glial cells to re-enter the cell cycle and produce transient-amplifying progenitors. These progenitors continue to proliferate as they migrate to the damaged area, where they ultimately give rise to new photoreceptors. Currently, there are two widely-used LIRD paradigms, each of which results in varying degrees of photoreceptor loss and corresponding differences in the regeneration response. As more genetic and pharmacological tools are available to test the role of individual genes of interest during regeneration, there is a need to develop a robust LIRD paradigm. Here we describe a LIRD protocol that results in widespread and consistent loss of both rod and cone photoreceptors in which we have combined the use of two previously established LIRD techniques. Furthermore, this protocol can be extended for use in pigmented animals, which eliminates the need to maintain transgenic lines of interest on the *albino* background for LIRD studies.

Protocol

All procedures described in this protocol were approved by the animal use committee at Wayne State University School of Medicine.

1. Dark Adaptation

1. Transfer ~10 adult *albino* or pigmented fish from the normal housing system into a dark enclosure. If available, use a dark enclosure that is built into the zebrafish housing module, which allows for normal water flow through the tank. (If such a system is not available, place the fish tank in a completely dark enclosure, making sure to aerate the fish with oxygen).
2. Keep the fish in the dark for 10 days. When feeding the animals or adding new fish to the dark box, make sure to move as quickly as possible to avoid exposing the fish to a long period of light.

2. UV Light Exposure

1. Make sure the power to the UV source is OFF. Remove the UV light filament from fluorescent stereomicroscope.
    1. Use an inverted 15 cm diameter glass Petri dish (or something of a similar 2 cm height) as a stand for the UV filament. Tape the Petri dish to the lab bench.

2. Tape the UV light filament to top of the inverted Petri dish. Arrange so that ~2 mm of the end of the filament overhangs the Petri dish.

2. Obtain a 250 ml glass beaker. Cover ½ of the bottom, sides, and back of the beaker with aluminum foil, making sure the "shiny" side of the foil faces the interior of the beaker. If the beaker is graduated, cover the worded half of the beaker with foil, leaving the clear half of the beaker exposed.
3. Fill the 250 ml beaker with 100 ml of water from the fish facility system.
4. Place the 250 ml beaker in a 4 L beaker. Fill the 4 L beaker with water until the water level is even with the 100 ml water line in the 250 ml beaker.
5. Add a maximum of 10 dark-treated animals to the 250 ml beaker. Cover the 250 ml beaker with a small piece of aluminum foil.
6. Place the entire beaker apparatus immediately adjacent to the UV filament. The filament should be touching the outside of the 4 L beaker and facing the exposed portion of the 250 ml beaker. Make sure that the 250 ml beaker is centered in the bottom of the 4 L beaker.
7. Position a large, opaque screen behind the 4L beaker, allowing for the animals to be exposed, but preventing any lab personnel from seeing the tip of the UV filament. WARNING: make sure this barrier is in place BEFORE turning on the power to the UV source.
8. Turn on the UV power source. Set timer for 30 min. Place whatever necessary warning labels to ensure that unsuspecting lab personnel do not accidentally expose themselves to UV radiation.
9. After 30 min, shut off the UV power source. Remove the 250 ml beaker with the fish. NOTE: if multiple rounds of UV exposure are required, let the power source cool down (~20 min) before repeating the exposure protocol. Make sure to replace the 100 ml of water with fresh system water for each exposure. The water in the 4 L beaker does not need to be replaced.

3. Halogen Lamp Light Exposure

1. Transfer the fish from 250 ml beaker into a 1.8 L clear acrylic fish tank. Fill the tank to the overflow mark.
2. Set up the halogen lamp light treatment area. Obtain four 250 W halogen utility lamps from a local hardware store. Facing two lamps in the same direction, arrange 29 cm apart on center. Arrange the other two lamps in a similar fashion.
    1. Place the second set of lamps so that they are facing the first set of lamps, leaving ~73 cm in between the two sets of lamps. This creates a rectangle-shaped light treatment area of 29 x 73 cm.

3. Obtain a small fan from a local hardware store. Place the fan equidistant between the two sets of lamps, just outside of the light treatment area.
4. Place two 1.8 L tanks full of water in the center of the light treatment area, equidistant between the two sets of lamps. The distance between the lamps and the outside wall of the tank should be ~29 cm. Note: even if only treating 10 fish in one 1.8 L tank, use this arrangement. Both tanks are needed to keep the water temperature of each tank within the appropriate range.
5. Place an oxygen aerator in each tank.
6. Cover each tank with clear acrylic lids, leaving a ~2 cm gap at the end closest to the fan. Arrange the fan so that it will blow air into this gap. Place a thermometer in one or both of the tanks.
7. Turn on the power to the lights, fan, and aerators. Maintain light treatment for up to 4 days. The fish should not be fed during the light treatment, as this will affect water quality and result in more stress to the animals.
8. Monitor temperature and water level on a daily basis. Maintain the temperature at 30-33 °C. If necessary, adjust the fan speed and/or the distance between the tanks and the lights.
    1. Top off each tank with system water as needed, usually daily.
    2. Always use healthy adult zebrafish (typically 6-9 months of age) to maintain a survival rate of near 100%. However, if a fish is found dead in the tank, remove it immediately and replace the water in the entire tank.

4. Tissue Collection

1. 48 hr after the onset of the halogen light treatment remove the fish from the treatment area.
    1. Prepare fresh ethanolic formaldehyde fixative (1 part 37% formaldehyde, 9 parts 100% ethanol).
    2. Euthanize zebrafish with an anesthetic overdose of 2-phenoxyethanol (0.4 mg/L).
    3. Transfer the euthanized zebrafish to a paper towel. Enucleate the eye using curved forceps.
    4. Place enucleated eyes in fixative and store O/N at 4 °C.

2. Cryoprotect the eyes.
    1. Wash the eyes in 5% sucrose 1x PBS for 30 min at room temperature, then replace with fresh 5% sucrose 1x PBS for 2 hr. Next, wash the eyes in 30% sucrose 1x PBS O/N at 4 °C. Wash the eyes in a 1:1 (equal portions) of tissue freezing medium and 30% sucrose 1x PBS O/N at 4 °C.

3. Embed the eyes in 100% tissue freezing medium and store at -80 °C. Orient the eyes so that cryosectioning of the tissue is performed on the dorsal/ventral axis.
4. Cryosection the tissue and place on glass slides. Warm slides for 2 hr at 55 °C. Store slides at -80 °C or immediately perform standard immunohistochemistry.
5. Perform standard immunohistochemistry on sectioned tissue and image with fluorescent microscopy[40,51].

Representative Results

The heretofore described light treatment protocol was compared to each individual method of LIRD. In dark-treated adult *albino* animals (**Figures 3-5**), the individual light treatments resulted in significant loss of rod (**Figure 3**) and cone (**Figure 4**) photoreceptors. However, both individual treatments primarily damaged photoreceptors in the dorsal half of the retina, leaving the ventral retina relatively protected from the light

treatments (**Figures 3** and **4**). In addition, compared with the halogen light treatment, the UV light treatment damaged more cone photoreceptors in the dorsal half of the retina (compare **Figures 4B** with **C**, respectively). Combining the UV and halogen light treatments resulted in significantly greater loss to both rod and cone photoreceptors throughout the retina, largely eliminating the neuroprotection of the ventral half of the retina (**Figures 3** and **4**). Compared with the individual LIRD methods, a corresponding increase in proliferation was also observed in the ventral retina when the combined light treatment protocol was used (**Figure 5**).

Pigmented animals are more resistant to LIRD due to retinal pigmented epithelium, which absorbs light and protects the photoreceptors from phototoxicity. As expected, dark-adapted pigmented animals were nearly completely resistant to the halogen light treatment alone (**Figures 6B, J,** and **N**). We found that 48 hr after light onset, rod photoreceptors were reduced in the dorsal retina, but not ventral retina (**Figures 6E, F,** and **Q**). Cone photoreceptor nuclei were present in normal numbers (**Figure 6R**). Compared with untreated retinas, halogen light treatment alone also did not increase Müller glial cell proliferation 48 hr after light onset (**Figures 7B** and **F**). In *albino* animals, UV light treatment alone resulted in slightly greater damage to both rod and cone photoreceptors in the dorsal retina (**Figures 3I** and **4I**). However, in pigmented animals, UV light treatment alone did not significantly reduce rod or cone cell number (**Figures 6C, G, K, O, Q,** and **R**). In addition, UV treatment alone only elicited a weak regenerative response in the dorsal half of the retina in pigmented animals (**Figure 7C**). In contrast to the individual LIRD results, combining the UV and halogen light treatments resulted in significantly greater photoreceptor damage and regenerative response in pigmented animals. Importantly, significant loss of both rods and cones was observed in both the dorsal and ventral retinas (**Figures 6D, H, L, P, Q,** and **R**). Compared with untreated and individual LIRD methods, a corresponding increase in proliferation was also observed in both dorsal and ventral halves of the retina when the combined light treatment protocol was used (**Figure 5**).

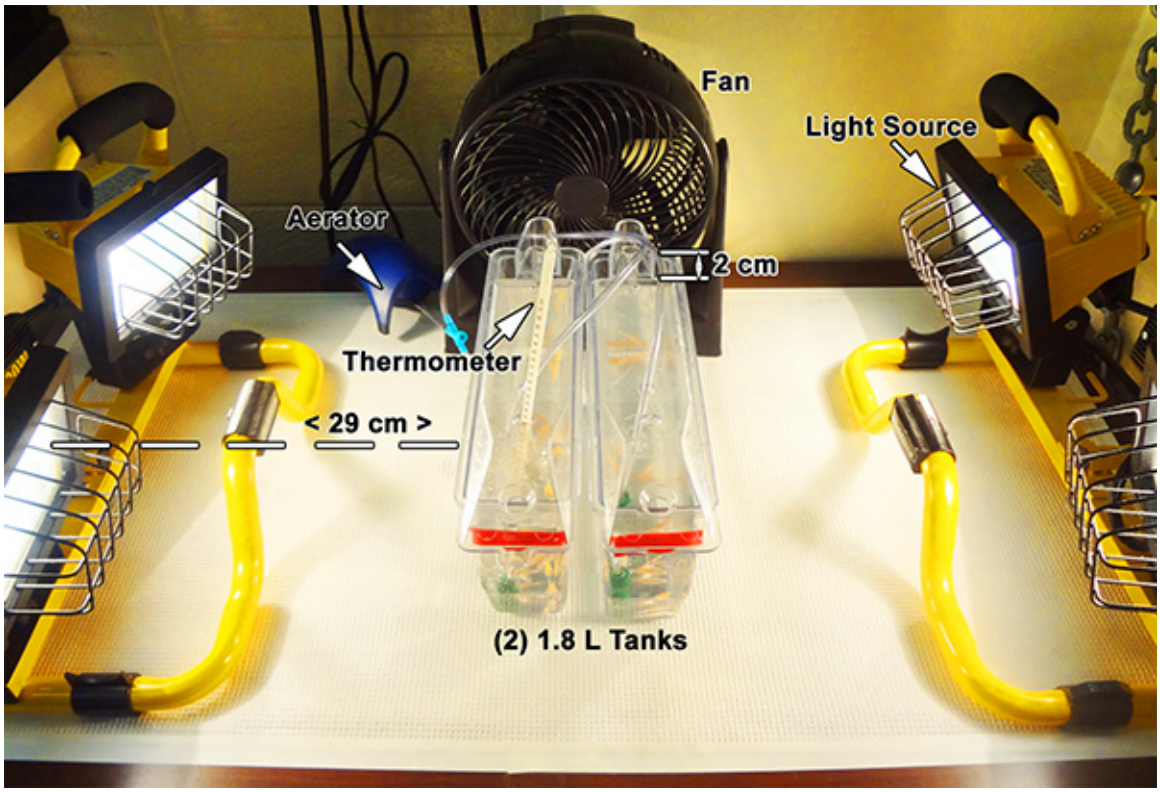

Figure 1. Photograph depicting the halogen light treatment setup. Two sets of four 250 W halogen lamps were spaced 29 cm on either side of two clear 1.8 L acrylic tanks. The aerator and tubing were placed so as not to obstruct the light. The lids of the tanks were cracked 2 cm to allow airflow from the fan. A thermometer was placed in one of the tanks for the temperature to be monitored. Click here to view larger image.

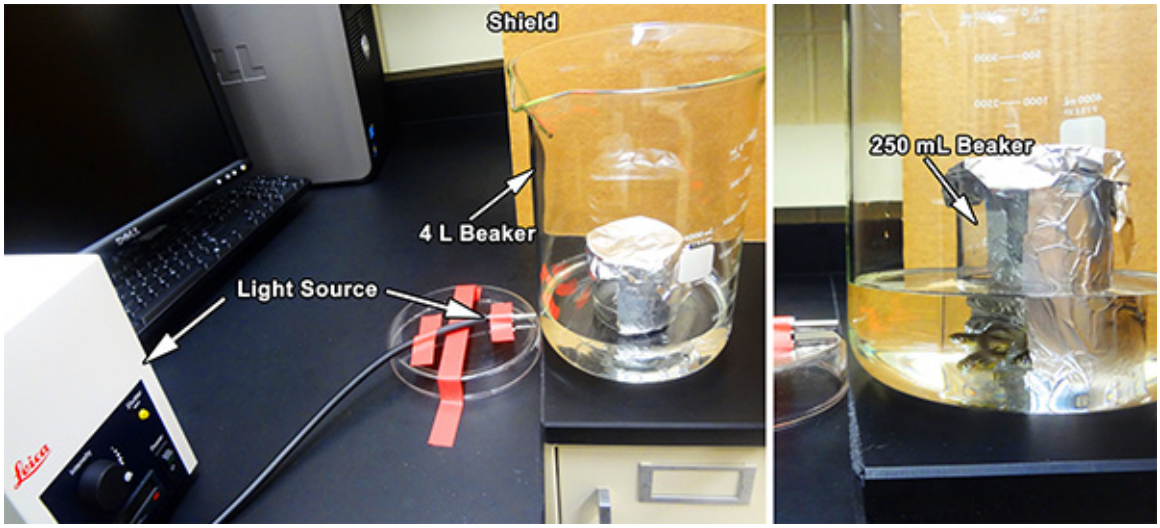

Figure 2. Photograph depicting the UV light treatment setup. The UV light source from a fluorescent stereomicroscope was securely fixed to a glass Petri dish ~2 cm off the bench top. A 250 ml glass beaker, partially wrapped in foil, was filled will 100 ml of system water. The fish were placed inside the 250 ml beaker. The 250 ml beaker was centered inside a 4 L beaker, which was filled with system water to the level of the water in the 250 ml beaker. The 4 L beaker was placed adjacent to the light filament centered at the 250 ml beaker. A large opaque shield was placed around the entire setup. Click here to view larger image.

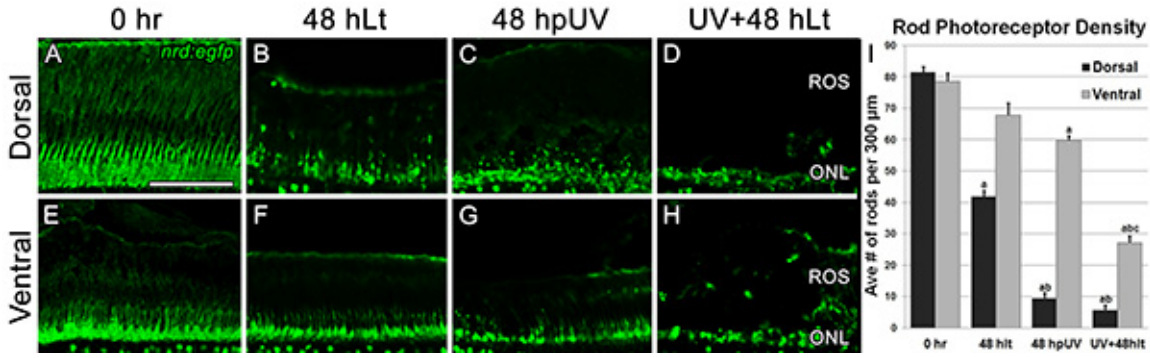

Figure 3. Rod photoreceptor loss in *albino* zebrafish following various light damage paradigms. Retinal sections from adult Tg(*nrd:egfp*)/*alb* immunolabeled with GFP to show rod photoreceptor density (green) in the dorsal (**A-D**) and ventral (**E-H**) halves of the retina. **A, E**) Prior to light onset (0 hr), EGFP-positive rod photoreceptor nuclei (ONL) and outer segments (ROS) can be visualized. **B, F**) At 48 hr following halogen light onset (hLt), truncated ROS and significantly fewer rod nuclei (**I**) were present in the dorsal, but not ventral retina. **C-G**) At 48 hr after 30 min of UV exposure (hpUV), significantly fewer rod nuclei were present in the dorsal and ventral retinas compared to 0 hr (**C, G**), but not 48 hLt treatments (**G, I**). **D-H**) 48 hr following onset of combined light treatment (30 min UV exposure followed by halogen treatment; UV+48 hLt), almost no ROS were present in dorsal or ventral retinas, in addition to a significant loss of rod photoreceptor nuclei (**I**). **I**) Quantification of rod photoreceptor nuclei across 300 µm of the central dorsal or ventral retina. Significant differences ($p \leq 0.05$) between groups are depicted as: "a" for different from 0 hr, "b" for different from 48 hLt and "c" for different from 48 hpUV. Scale bar in panel **A** represents 50 µm and applies to panels **A-H**. Click here to view larger image.

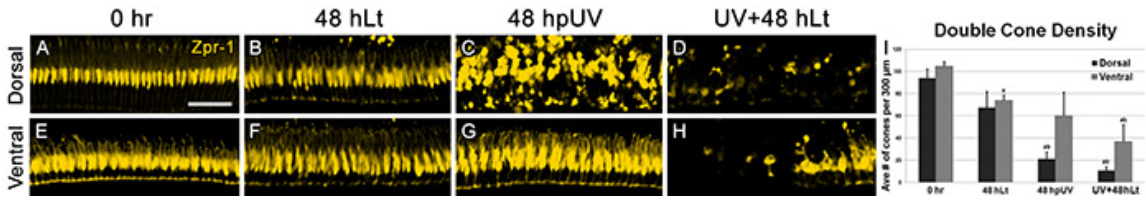

Figure 4. Double cone loss in *albino* zebrafish following various light damage paradigms. Retinal sections from adult *albino* zebrafish immunolabeled with Zpr-1 to show double cones (gold) in the dorsal (**A-D**) and ventral (**E-H**) halves of the retina following each of the light damage paradigms (48 hLt, 48 hpUV, and UV+48 hLt). **A**, **E**) At 0 hr, double cone nuclei were present in dorsal and ventral retinas. **B, F**) At 48 hLt, significantly fewer double cones were present in the ventral retina only (**F, I**). C-G) At 48 hpUV, significantly fewer double cones (**I**) and a large amount of debris was present in the dorsal retina only (**C**), while no changes were observed in the ventral retina (**G**)**. D-H**) At UV+48 hLt, significantly fewer double cones (**I**) and very little debris was present in dorsal and ventral retinas. I) Quantification of double cones across 300 µm of the central dorsal or ventral retina. Significant differences ($p \leq 0.05$) between groups are depicted as, "a" for different from 0 hr, "b" for different from 48 hLt and "c" for different from 48 hpUV. Scale bar in panel **A** represents 100 µm and applies to panels **A-H**. Click here to view larger image.

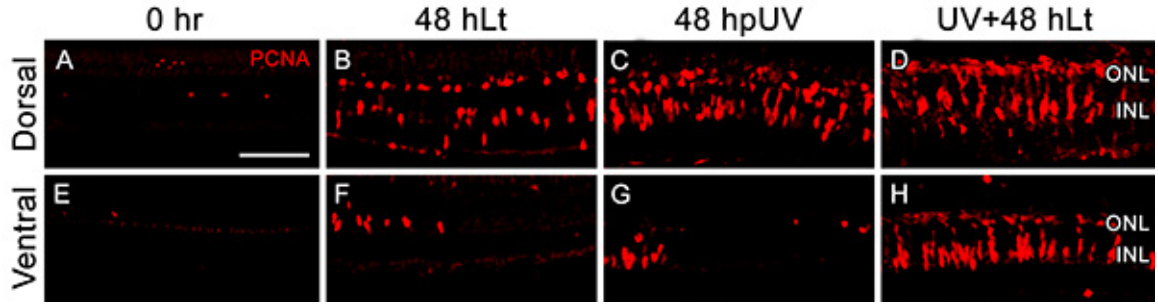

Figure 5. Changes is proliferation response following various light damage paradigms. Retinal sections from adult *albino* zebrafish immunolabeled with PCNA to show cell proliferation (red) in the dorsal (**A-D**) and ventral (**E-H**) halves of the retina following each of the light damage paradigms (48 hLt, 48 hpUV, and UV+48 hLt). **A-E**) At 0 hr, proliferation was absent from the inner nuclear layer (**INL**). **B, F**) At 48 hLt, single progenitor cells were present in the INL across the dorsal retina and in a small portion of the ventral retina. **C-G**) At 48 hpUV, columns of progenitor cells were present in the dorsal retina, while only single progenitor cells were present in a small portion of the ventral retina. **D, H**) At UV+48 hLt, large columns of progenitor cells were present across the dorsal and ventral retina. Scale bar in panel **A** represents 100 µm and applies to panels **A-H**. Click here to view larger image.

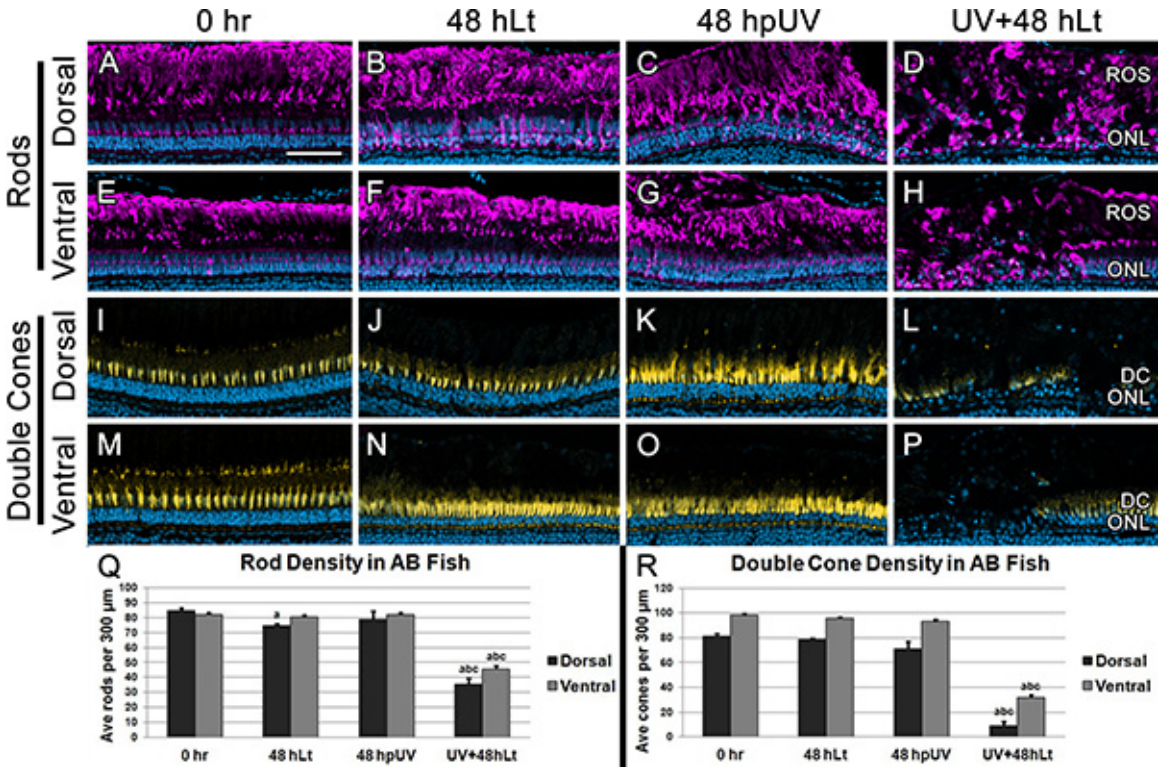

Figure 6. Rod and cone photoreceptor loss in pigmented zebrafish following various light damage paradigms. Retinal sections from adult wild-type (AB) zebrafish stained with TO-PRO-3 to show all nuclei (**A-P**; blue) and immunolabeled with Zpr-3 to show rod outer segments (**A-H**; magenta) or Zpr-1 to double cones (**I-P**; gold) in the dorsal (**A-D, I-J**) and ventral (**E-H, M-P**) halves of the retina following each of the light damage paradigms (48 hLt, 48 hpUV, and UV+48 hLt). **A-H**) Significant decreases in rod photoreceptor nuclei were present in the dorsal retina at 48 hLt (**B, Q**) and in the dorsal and ventral retina at UV+48 hLt (**D, H, Q**). **I-P**) Although cone nuclei were hypertrophied in the dorsal retina at 48 hpUV (**K**), no significant decreases in double cone nuclei were present at 48 hLt or 48 hpUV (**J-K, N-O, R**). Significant decreases in cone nuclei were present in the dorsal and ventral retina at UV+48 hLt (**L, P, R**). **Q-R**) Quantification of rod photoreceptor and double cone nuclei across 300 µm of the central dorsal or ventral retina. Significant differences ($p \leq 0.05$) between groups are depicted as, "a" for different from 0 hr, "b" for different from 48 hLt and "c" for different from 48 hpUV. Scale bar in panel **A** represents 200 µm and applies to panels **A-H**. Click here to view larger image.

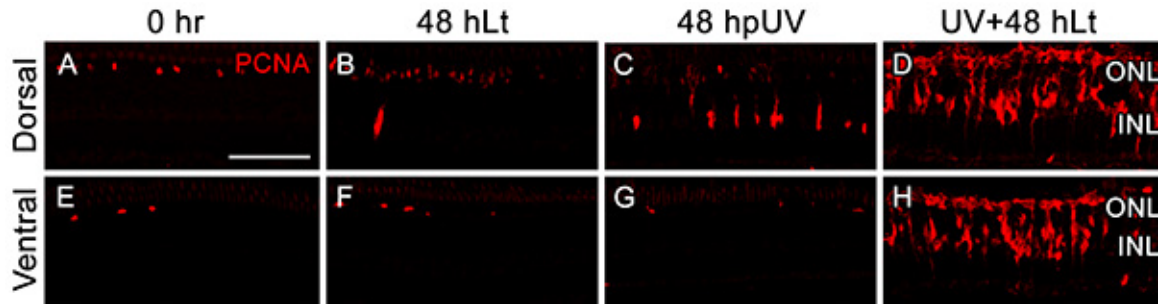

Figure 7. Proliferation response in pigmented zebrafish following various light damage paradigms. Retinal sections from adult wild-type (AB) zebrafish immunolabeled with PCNA to show cell proliferation (red) in the dorsal (**A-D**) and ventral (**E-H**) halves of the retina following each of the light damage paradigms (48 hLt, 48 hpUV, and UV+48 hLt). **A-D**) In the dorsal retina, only a few single progenitor cells were present in the INL at 48 hLt and 48 hpUV (**B, C**), while columns of progenitor cells were present at UV+48 hLt (**D**). **E-H**) In the ventral retina, no changes in proliferation were observed except at UV+48 hLt (**H**), at which time columns of progenitor cells were present. Scale bar in panel **A** represents 100 µm and applies to panels **A-H**. Click here to view larger image.

Discussion

Here we show that combining a short UV exposure with a continual bright light exposure results in widespread photoreceptor loss and a robust regeneration response. Compared with the individual LIRD methods, this combined method is also the most effective protocol to damage both rods and cones in both halves of the retina. Importantly, this treatment is effective in pigmented animals as well as *albino* animals.

Although we provide evidence that the combined protocol results in more widespread and consistent damage compared with the individual LIRD methods, scientific considerations should be discussed before selecting a LIRD paradigm. Our data suggest that the combined method should be utilized when the entire retinal tissue is collected for analysis (*i.e.* proteomics, real-time PCR). We found that the combined method had the greatest lesion size, followed by the halogen exposure, and finally the UV exposure. We suggest that the combined method also be used when investigating a gene or pathway that could differentially effect rod and cone regeneration. If this is not possible for practical reasons, our data suggest that the UV exposure is the next best method for these studies, if the analysis is limited to the central dorsal retina.

Practical considerations are also warranted when selecting the appropriate LIRD method. The first concern is the cost of the necessary equipment. All the protocols require a prolonged period of dark adaptation (data not shown). A self-contained dark enclosure that is built into the zebrafish housing module is extremely convenient, but not necessary. Even a cardboard box could be used, if care is taken to tape or seal the seams, and the water parameters are monitored. The halogen-based LIRD method is extremely economical and convenient. All items can be purchased for under $100 US at a local hardware store and set up takes just a few minutes. The only practical disadvantage of this method is the inconvenience of working in the same room as the light treatment. The lights generate a good amount of heat when running continuously for 4 days and the light intensity is both distracting and potentially damaging to human vision. The UV and combined methods require a UV source, which is not available to every laboratory. In addition, great care should be taken to ensure the safety of the lab members when performing a UV light treatment. Thus, while it is more convenient to have the halogen light apparatus in an isolated space, the safety concerns of the UV treatment necessitate such a space. This becomes an additional inconvenience if the UV source used for the light treatment is also typically used for a fluorescent microscope housed in a "microscope room" or imaging core. Performing multiple rounds of UV treatments for control and experimental groups of animals would necessitate shutting down the microscope room for an extensive length of time.

Perhaps the greatest advantage to using the combined method is the ability to perform retinal regeneration studies on pigmented animals. We show that neither individual method results in significant loss of rods and cones in the dorsal and ventral retina. In contrast, the combined LIRD paradigm results in similar levels of widespread rod and cone loss in both pigmented and *albino* animals. The ability to apply this LIRD method to pigmented animals saves the researcher significant space, time, and per diem animal care costs (if any), as it eliminates the need to generate and maintain transgenic lines of interest on the *albino* background for retinal regeneration studies. Together, we feel that the scientific and practical advantages outweigh the potential inconveniences of this improved LIRD method.

Disclosures

The authors have nothing to disclose.

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References

1. Bernardos, R. L., Barthel, L. K., Meyers, J. R. & Raymond, P. A. Late-stage neuronal progenitors in the retina are radial Muller glia that function as retinal stem cells. *J. Neurosci.* **27**, 7028-7040 (2007).
2. Fausett, B. V. & Goldman, D. A role for alpha1 tubulin-expressing Muller glia in regeneration of the injured zebrafish retina. *J. Neurosci.* **26**, 6303-6313 (2006).
3. Fimbel, S. M., Montgomery, J. E., Burket, C. T. & Hyde, D. R. Regeneration of inner retinal neurons after intravitreal injection of ouabain in zebrafish. *J. Neurosci.* **27**, 1712-1724 (2007).
4. Sherpa, T. *et al.* Ganglion cell regeneration following whole-retina destruction in zebrafish. *Dev. Neurobiol.* **68**, 166-181 (2008).
5. Thummel, R. *et al.* Pax6a and Pax6b are required at different points in neuronal progenitor cell proliferation during zebrafish photoreceptor regeneration. *Exp. Eye Res.* **90**, 572-582 (2010).
6. Vihtelic, T. S. & Hyde, D. R. Light-induced rod and cone cell death and regeneration in the adult albino zebrafish (*Danio rerio*) retina. *J. Neurobiol.* **44**, 289-307 (2000).
7. Vihtelic, T. S., Soverly, J. E., Kassen, S. C. & Hyde, D. R. Retinal regional differences in photoreceptor cell death and regeneration in light-lesioned albino zebrafish. *Exp. Eye Res.* **82**, 558-575 (2006).
8. Yurco, P. & Cameron, D. A. Responses of Muller glia to retinal injury in adult zebrafish. *Vision Res.* **45**, 991-1002 (2005).
9. Kassen, S. C. *et al.* Time course analysis of gene expression during light-induced photoreceptor cell death and regeneration in albino zebrafish. *Dev. Neurobiol.* **67**, 1009-1031 (2007).
10. Raymond, P. A., Barthel, L. K., Bernardos, R. L. & Perkowski, J. J. Molecular characterization of retinal stem cells and their niches in adult zebrafish. *BMC Dev. Biol.* **6**, 36 (2006).
11. Thummel, R. *et al.* Characterization of Muller glia and neuronal progenitors during adult zebrafish retinal regeneration. *Exp. Eye Res.* **87**, 433-444 (2008).
12. Thummel, R., Kassen, S. C., Montgomery, J. E., Enright, J. M. & Hyde, D. R. Inhibition of Muller glial cell division blocks regeneration of the light-damaged zebrafish retina. *Dev. Neurobiol.* **68**, 392-408 (2008).
13. Montgomery, J. E., Parsons, M. J. & Hyde, D. R. A novel model of retinal ablation demonstrates that the extent of rod cell death regulates the origin of the regenerated zebrafish rod photoreceptors. *J. Comp. Neurol.* **518**, 800-814 (2010).
14. Morris, A. C., Scholz, T. L., Brockerhoff, S. E. & Fadool, J. M. Genetic dissection reveals two separate pathways for rod and cone regeneration in the teleost retina. *Dev. Neurobiol.* **68**, 605-619 (2008).

15. Bignami, A. & Dahl, D. The radial glia of Muller in the rat retina and their response to injury. An immunofluorescence study with antibodies to the glial fibrillary acidic (GFA) protein. *Exp. Eye Res.* **28**, 63-69 (1979).
16. Bringmann, A. *et al.* Muller cells in the healthy and diseased retina. *Prog. Retin. Eye Res.* **25**, 397-424, doi:10.1016/j.preteyeres.2006.05.003 (2006).
17. Bringmann, A. & Wiedemann, P. Muller glial cells in retinal disease. *Ophthalmologica.* **227**, 1-19, doi:10.1159/000328979 (2012).
18. Eisenfeld, A. J., Bunt-Milam, A. H. & Sarthy, P. V. Muller cell expression of glial fibrillary acidic protein after genetic and experimental photoreceptor degeneration in the rat retina. *Invest. Ophthalmol. Vis. Sci.* **25**, 1321-1328 (1984).
19. Anderson, D. H., Guerin, C. J., Erickson, P. A., Stern, W. H. & Fisher, S. K. Morphological recovery in the reattached retina. *Invest. Ophthalmol. Vis. Sci.* **27**, 168-183 (1986).
20. Bruce, A. E., Oates, A. C., Prince, V. E. & Ho, R. K. Additional hox clusters in the zebrafish: divergent expression patterns belie equivalent activities of duplicate hoxB5 genes. *Evol. Dev.* **3**, 127-144 (2001).
21. Fawcett, J. W. & Asher, R. A. The glial scar and central nervous system repair. *Brain Res. Bull.* **49**, 377-391 (1999).
22. Lewis, G. P. & Fisher, S. K. Muller cell outgrowth after retinal detachment: association with cone photoreceptors. *Invest. Ophthalmol. Vis. Sci.* **41**, 1542-1545 (2000).
23. Fischer, A. J. & Bongini, R. Turning Muller glia into neural progenitors in the retina. *Mol. Neurobiol.* **42**, 199-209, doi:10.1007/s12035-010-8152-2 (2010).
24. Karl, M. O. & Reh, T. A. Regenerative medicine for retinal diseases: activating endogenous repair mechanisms. *Trends Mol. Med.* **16**, 193-202, doi:10.1016/j.molmed.2010.02.003 (2010).
25. Close, J. L., Liu, J., Gumuscu, B. & Reh, T. A. Epidermal growth factor receptor expression regulates proliferation in the postnatal rat retina. *Glia.* **54**, 94-104, doi:10.1002/glia.20361 (2006).
26. Das, A. V. *et al.* Neural stem cell properties of Muller glia in the mammalian retina: regulation by Notch and Wnt signaling. *Dev. Biol.* **299**, 283-302, doi:10.1016/j.ydbio.2006.07.029 (2006).
27. Ikeda, T. & Puro, D. G. Regulation of retinal glial cell proliferation by antiproliferative molecules. *Exp. Eye Res.* **60**, 435-443 (1995).
28. Liu, B. *et al.* Wnt signaling promotes muller cell proliferation and survival after injury. *Invest. Ophthalmol. Vis. Sci.* **54**, 444-453, doi:10.1167/iovs.12-10774 (2013).
29. Osakada, F. *et al.* Wnt signaling promotes regeneration in the retina of adult mammals. *J. Neurosci.* **27**, 4210-4219, doi:10.1523/JNEUROSCI.4193-06.2007 (2007).
30. Wan, J. *et al.* Preferential regeneration of photoreceptor from Muller glia after retinal degeneration in adult rat. *Vision Res.* **48**, 223-234 (2008).
31. Wan, J., Zheng, H., Xiao, H. L., She, Z. J. & Zhou, G. M. Sonic hedgehog promotes stem-cell potential of Muller glia in the mammalian retina. *Biochem. Biophys. Res. Commun.* **363**, 347-354 (2007).
32. Roesch, K. *et al.* The transcriptome of retinal Muller glial cells. *J. Comp. Neurol.* **509**, 225-238, doi:10.1002/cne.21730 (2008).
33. Chang, G. Q., Hao, Y. & Wong, F. Apoptosis: final common pathway of photoreceptor death in rd, rds, and rhodopsin mutant mice. *Neuron.* **11**, 595-605 (1993).
34. Marc, R. E. *et al.* Extreme retinal remodeling triggered by light damage: implications for age related macular degeneration. *Mol. Vis.* **14**, 782-806 (2008).
35. Portera-Cailliau, C., Sung, C. H., Nathans, J. & Adler, R. Apoptotic photoreceptor cell death in mouse models of retinitis pigmentosa. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 974-978 (1994).
36. Noell, W. K., Walker, V. S., Kang, B. S. & Berman, S. Retinal damage by light in rats. *Invest. Ophthalmol.* **5**, 450-473 (1966).
37. Wenzel, A., Grimm, C., Samardzija, M. & Reme, C. E. Molecular mechanisms of light-induced photoreceptor apoptosis and neuroprotection for retinal degeneration. *Prog. Retin. Eye Res.* **24**, 275-306 (2005).
38. Cicerone, C. M. Cones survive rods in the light-damaged eye of the albino rat. *Science.* **194**, 1183-1185 (1976).
39. La Vail, M. M. Survival of some photoreceptor cells in albino rats following long-term exposure to continuous light. *Invest. Ophthalmol.* **15**, 64-70 (1976).
40. Thomas, J. L., Nelson, C. M., Luo, X., Hyde, D. R. & Thummel, R. Characterization of multiple light damage paradigms reveals regional differences in photoreceptor loss. *Exp. Eye Res.* **97**, 105-116, doi:10.1016/j.exer.2012.02.004 (2012).
41. Qin, Z. *et al.* FGF signaling regulates rod photoreceptor cell maintenance and regeneration in zebrafish. *Exp. Eye Res.* **93**, 726-734 (2011).
42. Craig, S. E. *et al.* The zebrafish galectin Drgal1-l2 is expressed by proliferating Muller glia and photoreceptor progenitors and regulates the regeneration of rod photoreceptors. *Invest. Ophthalmol. Vis. Sci.* **51**, 3244-3252 (2010).
43. Morris, A. C., Schroeter, E. H., Bilotta, J., Wong, R. O. & Fadool, J. M. Cone survival despite rod degeneration in XOPS-mCFP transgenic zebrafish. *Invest. Ophthalmol. Vis. Sci.* **46**, 4762-4771 (2005).
44. Nelson, C. M. & Hyde, D. R. Muller glia as a source of neuronal progenitor cells to regenerate the damaged zebrafish retina. *Adv. Exp. Med. Biol.* **723**, 425-430, doi:10.1007/978-1-4614-0631-0_54 (2012).
45. Powell, C., Elsaeidi, F. & Goldman, D. Injury-dependent Muller glia and ganglion cell reprogramming during tissue regeneration requires Apobec2a and Apobec2b. *J. Neurosci.* **32**, 1096-1109, doi:10.1523/JNEUROSCI.5603-11.2012 (2012).
46. Ramachandran, R., Reifler, A., Wan, J. & Goldman, D. Application of Cre-loxP recombination for lineage tracing of adult zebrafish retinal stem cells. *Methods Mol. Biol.* **884**, 129-140, doi:10.1007/978-1-61779-848-1_8 (2012).
47. Ramachandran, R., Zhao, X. F. & Goldman, D. Ascl1a/Dkk/beta-catenin signaling pathway is necessary and glycogen synthase kinase-3beta inhibition is sufficient for zebrafish retina regeneration. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 15858-15863 (2011).
48. Ramachandran, R., Zhao, X. F. & Goldman, D. Insm1a-mediated gene repression is essential for the formation and differentiation of Muller glia-derived progenitors in the injured retina. *Nat. Cell Biol.* **14**, 1013-1023, doi:10.1038/ncb2586 (2012).
49. Wan, J., Ramachandran, R. & Goldman, D. HB-EGF is necessary and sufficient for Muller glia dedifferentiation and retina regeneration. *Dev. Cell.* **22**, 334-347, doi:10.1016/j.devcel.2011.11.020 (2012).
50. Morris, A. C., Forbes-Osborne, M. A., Pillai, L. S. & Fadool, J. M. Microarray analysis of XOPS-mCFP zebrafish retina identifies genes associated with rod photoreceptor degeneration and regeneration. *Invest. Ophthalmol. Vis. Sci.* **52**, 2255-2266 (2011).
51. Thummel, R., Bailey, T. J. & Hyde, D. R. *In vivo* electroporation of morpholinos into the adult zebrafish retina. *J. Vis. Exp.* (58), e3603, doi:10.3791/3603 (2011).

