Neuroprotection in the Juvenile Rat Model of Light-Induced Retinopathy: Evidence Suggesting a Role for FGF-2 and CNTF

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Purpose. In a former study, it was demonstrated that the retina of juvenile Sprague-Dawley (SD) rat has a remarkable intrinsic resistance to light-induced retinopathy compared with the adult retina. The purpose of the present study was to investigate the cellular and molecular mechanisms underlying this endogenous resistance to light-induced damage.

Methods. Juvenile SD rats were exposed for 6 (from P14 to P20) or 14 (from P14 to P28) days to a bright, cyclic, luminous environment of 10,000 lux. Retinal histology was examined immediately after exposure to light or at 2 months of age, and photoreceptor cell death was quantified by measuring the thickness of the outer nuclear layer (ONL) and by TUNEL assays. Changes in protein levels and cellular localization of fibroblast growth factor (FGF)-2, ciliary neurotrophic factor (CNTF), and brain-derived neurotrophic factor (BDNF) were determined by Western blot analysis and retinal immunohistochemistry, respectively.

Results. The data demonstrate that although the rate of photoreceptor loss was different after 6 and 14 days of exposure to light, similar ONL thickness was reached at 2 months of age—that is, 4 to 5 weeks after exposure to light. A large number of TUNEL-positive photoreceptors was visualized immediately after 6 and 14 days of exposure to light, reflecting the intense cell death that was occurring in the ONL. Western blot analysis showed that exposure to light induced a strong upregulation of the neurotrophic factors FGF-2 and CNTF in juvenile retinas, whereas no change in BDNF protein expression was noted. Of interest, after exposure to light, endogenous FGF-2 and CNTF were selectively upregulated in Müller cells.

Conclusions. The results show that endogenous expression of FGF-2 and CNTF by Müller glia may play a role in protecting the juvenile retina from light-induced damage. (*Invest Ophthalmol Vis Sci.* 2007;48:2311–2320) DOI:10.1167/iovs.06-1205

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Bright exposure to light leads to severe retinal degeneration in adult albino rats.[1–9] The sequence of cellular events leading to photoreceptor degeneration is well established.[10] Progressive electrophysiological[2,3,11,12] and histologic deficits[10,13–16] are observed as the duration of exposure increases. In extreme cases, there is complete destruction of the photoreceptor layer, whereas the inner layers of the retina remain intact.[10,13,15,16] In contrast, we have recently demonstrated that the retina of juvenile rat is relatively resistant to light-induced damage during the first 30 postnatal days.[17] The exact molecular mechanisms behind this endogenous resistance to light-induced degeneration remain to be explored.

Neurotrophic factors are potent survival molecules that protect retinal cells from death induced by several stimuli.[18] In animal models of retinal degeneration, photoreceptor cell death is delayed after intravitreous injection of neurotrophic factors such as brain-derived neurotrophic factor (BDNF),[19,20] ciliary neurotrophic factor (CNTF),[20,21] fibroblast growth factor (FGF)-2,[20,22,23] or glia-derived neurotrophic factor (GDNF).[24] Similarly, in light-induced retinal degeneration, photoreceptors can be protected by exogenous BDNF.[25–27] Moreover, intravitreous administration of pigment epithelium-derived factor (PEDF),[28,29] FGF-2,[27,29–31] or lens epithelium–derived growth factor (LEDGF)[32] also rescued photoreceptors after exposure to light. In adult retinas, the levels of some neurotrophic factors, including FGF-2 and CNTF, are upregulated after mechanical injury[33–35] and light-induced retinal degeneration.[22,36,37] Photoreceptors are protected from light-induced damage by local injury[31] or optic nerve axotomy.[38,39] Thus, the upregulation of endogenous neurotrophic factors may be a natural protective mechanism for adult photoreceptors.

Little is known about endogenous neurotrophic factor expression in the light-injured, developing retina. Neurotrophic factors show large variations during postnatal life.[33] For example, BDNF is strongly upregulated after eyelid opening (at P14) and reaches maximum levels at 1 month of age.[40] In addition, mechanical injury that occurs early after birth induces changes in neurotrophin mRNA levels, which are sustained for at least the first 6 months of age.[33] In light of these results, we hypothesized that variations in endogenous retinal trophic factors explain the resistance to light-induced damage that we have reported in the juvenile rat retina (<30 days of age).[17]

Therefore, the purpose of our study was to explore further the molecular mechanisms underlying the endogenous resistance to light that we observed in juvenile versus adult rat retinas. Our results indicate that after exposure to light, the juvenile rat retina showed enhanced FGF-2 and CNTF protein expression, both factors that may contribute to the neuroprotection of retinal tissue. Immunohistological analysis demonstrated that upregulation of both neurotrophic factors occurred selectively in Müller cells.

METHODS

Animals

All the procedures were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and also with the guidelines of the Canadian Council on Animal Care for the use of experimental animals. The procedures were approved by the McGill University-Montreal Children's Hospital Animal Care Committee. Pregnant female Sprague-Dawley rats (Charles River Laboratories, St-Constant, QC, Canada) were obtained at 15 days of gestation and kept under a normal cyclic (12-hour dark-light [12D-12L]) light environment of 80 lux. After birth, the pups were maintained at 80 lux until postnatal day (P)14. They were then exposed for 6 (from P14 to P20) or 14 (from P14 to P28) consecutive days to a cyclic bright light (10,000 lux; 12D-12L), using a method previously reported by us.[17] Control animals were raised in the normal cyclic light environment of the animal care facility.

Analysis of Photoreceptor Cell Death

Rats were euthanatized either immediately after the offset of exposure to light (i.e., in the evening) or at 2 months of age and were perfused intracardially with 4% paraformaldehyde (PFA) in 0.2 M phosphate buffer (PB; pH 7.4). The left eyes were kept for retinal histology, whereas the right eyes were used for TUNEL staining and also for the immunohistochemistry, to detect endogenous levels of the neurotrophic factors FGF-2 and CNTF ($n = 3$ for each regimen of exposure).

Retinal Histology. After the removal of the cornea and lens, eye cups were postfixed for 2 hours in 4% PFA solution in 0.2 M PB (pH 7.4). After a 4-hour fixation period in 1% osmium tetroxide and sequential dehydration in ethanol, the eyes were embedded in Epon resin (Mecalab; Montreal, QC, Canada). Retinal sections of 0.7 μm were cut, mounted on glass slides and stained with 0.1% toluidine blue. The thickness of the retinal layers was measured on seven different retinal sections in the superior and the inferior hemispheres (Image-Pro Plus Imaging Software; Media Cybernetics, MD). In both cases, retinal pictures were taken in the area of greatest regional susceptibility, localized between 0.5 and 2.0 mm from the optic nerve head.[41] On each section, retinal layer thicknesses were measured in five randomly chosen areas.

TUNEL Assay. The anterior structures of the eye were removed as described, and the eye cups were immersed for 2 hours in 4% PFA in 0.2 M PB (pH 7.4) and were cryoprotected in graded sucrose solutions (10%–30% in PB) at 4°C. The eyes were embedded in optimal cutting temperature (OCT) compound (Tissue-Tek; Miles Laboratories, Elkhart, IN) and frozen in a 2-methylbutane/liquid nitrogen bath. Cryosections (14–16 μm thick) were collected on gelatin-coated slides on which TUNEL assays were performed with an apoptosis detection kit (ApopTag Fluorescein In Situ Apoptosis Detection; Chemicon, Temecula, CA). TUNEL-positive cells were counted on the entire area of each retinal section. Retinal histology and fluorescent TUNEL staining were examined by microscope (Axioskop 2 Plus; Carl Zeiss Canada, Kirkland, QC, Canada), and images were captured with a charge-coupled device (CCD) video camera (Retiga, Qimaging, Burnaby, BC, Canada) and analyzed (Northern Eclipse software; Empix Imaging, Inc., Mississauga, ON, Canada).

Retinal Immunohistochemistry

Sections were incubated in 3% normal goat serum, 0.3% Triton X-100 (Sigma-Aldrich, St. Louis, MO) in 0.1 M PBS (pH 7.4) for 30 minutes at room temperature to block nonspecific binding. Retinal sections were incubated overnight at 4°C with the following primary antibodies: monoclonal anti-human FGF-2 (type II, clone bFM-2, 10 μg/mL; Upstate Biotechnology, Lake Placid, NY), monoclonal anti-mouse CNTF (5 μg/mL; Chemicon), polyclonal anti-rabbit GFAP (1:100; Sigma-Aldrich) and polyclonal anti-rabbit CRALBP (1:1000; generous gift from John Saari, University of Washington, Seattle, WA). Sections were then incubated with the appropriate secondary antibody for 1 hour at room temperature, washed in PBS, and mounted in an antifade reagent (SlowFade; Invitrogen-Molecular Probes, Eugene, OR). Fluorescence was observed by microscope (Axioskop 2 Plus; Carl Zeiss Canada), and images were analyzed (Northern Eclipse software; Empix Imaging, Inc.).

Western Blot Analysis

Retinas from exposed and age-matched control rats ($n = 3$ control; $n = 4$ for each regimen of exposure to light) were rapidly extracted and independently homogenized with an electric pestle (Kontes, Vineland, NJ) in ice-cold lysis buffer (0.1% sodium dodecyl sulfate, 20 mM Tris [pH 8.0], 135 mM sodium chloride, 1% NP-40, 10% glycerol supplemented with protease inhibitors). Homogenates from right and left retinas from each animal were pooled. Retinal lysates were incubated on ice for 30 minutes and then centrifuged at 14,000 rpm for 15 minutes, after which the supernatant was collected. Protein concentration was determined by the Lowry method (Bio-Rad Life Science, Mississauga, ON, Canada). For each sample, 100 μg of protein was resolved by electrophoresis on 15% sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) and transferred to nitrocellulose membranes (Bio-Rad Life Science). Blots were incubated in 10 mM Tris (pH 8.0), 150 mM NaCl, 0.2% Tween 20 (TBST), and 5% lyophilized skim milk for 1 hour at room temperature, to block nonspecific signals. Membranes were incubated overnight at 4°C with the following primary antibodies: monoclonal anti-human FGF-2 (type II, clone bFM-2, 2.5 μg/mL; Upstate Biotechnology), monoclonal anti-mouse CNTF (5 μg/mL; Chemicon), and monoclonal anti-human BDNF (anti-human BDNF pAb, 5 μg/mL; Promega Corp., Madison, WI). Membranes were washed with TBST and then incubated, for 1 hour at room temperature, with anti-mouse (Amersham Pharmacia, Baie d'Urfé, QC, Canada) or anti-chicken (Promega Corp.) peroxidase-linked secondary antibodies. Protein signals were detected with a chemiluminescent reagent (ECL; GE Healthcare, Piscataway, NJ) followed by exposure of membranes to autoradiograph film (X-OMAT; Eastman Kodak, Rochester, NY) imaging film. Membranes were incubated in a stripping solution (200 mM glycine [pH 2.8], 500 mM NaCl and 0.7% β-mercaptoethanol) at 55°C for 1 hour and then reprobed with an anti γ-actin polyclonal antibody (1:10,000; generous gift from J. Chloe Bulinski, Columbia University, New York, NY) followed by incubation with an anti-rabbit peroxidase-linked secondary antibody (Amersham Pharmacia Biotech). The γ-actin protein was chosen as a reference instead of β-actin, because expression of the latter changes dramatically during development.[42,43] Densitometric analysis was performed (BioDoc Analyze 1.0 software; Biometra, Göttingen, Germany) on scanned autoradiographic films obtained from a series of three independent Western blots, each performed with retinal samples from distinct experimental groups. The densitometric values obtained for each neurotrophic factor were normalized with respect to γ-actin level in the same blot.

Data Analysis

Statistical analyses were performed by using Student's *t*-test or one-way ANOVA followed by the Tukey post hoc test (Instate Software; GraphPad Inc., San Diego, CA).

RESULTS

Histologic analysis of normal retinas during postnatal development showed that the overall thickness of the retina decreased significantly until it reached its final size at adulthood (Fig. 1; P14: 270.7 ± 16.7 μm vs. 2 months: 220.3 ± 9.7 μm; $P < 0.05$, Student's *t*-test). The thickness of the outer nuclear layer (ONL) also decreased gradually with maturation. At P20 (Fig. 1B) and P28 (Fig. 1C), the thickness of the ONL was only 88% and 86% ($P < 0.05$; ANOVA) of the ONL at P14 (Fig. 1A) compared with 67% at P60 (Fig 1D; $P < 0.001$; ANOVA).

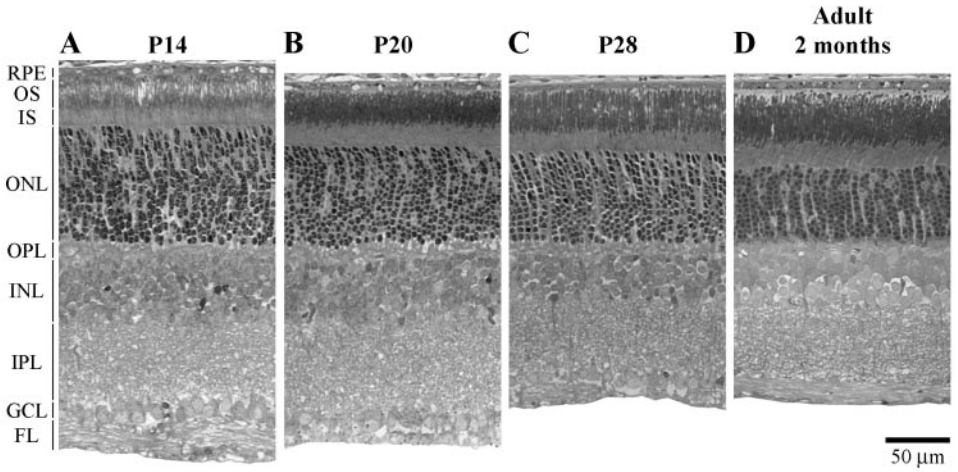


FIGURE 1. Representative photomicrographs of retinas obtained from juvenile rats at P14 (**A**), P20 (**B**), P28 (**C**) and 2 months (**D**), illustrating the normal development of the retina. Note the progressive thinning of the retina between P14 and 2 months. A reduction of the ONL thickness was most obvious between P14 and P20 and between P28 and 2 months. RPE, retinal pigmented epithelium; OS, outer segments; IS, inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; FL, nerve fiber layer. Scale bar, 50 μm.

To establish the time-course of light-induced damage, the retinas of juvenile rats were harvested immediately after exposure or 2 months after a 6-day (group P14–P20) or a 14-day (group P14–P28) exposure to bright light (10,000 lux). Immediately after the 6-day exposure regimen (P14–P20; Fig. 2B), the thickness of the ONL was reduced to 74% of that at P20 (Fig. 2A; $P < 0.05$) and further decreased to 51% (Fig. 2C; $P < 0.01$) at P60. In contrast, retinas from adult rats (60–90 days old) exposed for 6 days to bright light showed a complete loss of the photoreceptor layer (Fig. 2D). Similarly, after 14 days of exposure to light (P14–P28), the thickness of the ONL of the juvenile rat retinas significantly decreased to 46% (Fig. 2F; $P < 0.001$) of the P28 control value (Fig. 2E). This reduction seemed to be stable between P28 and 2 months of age. In

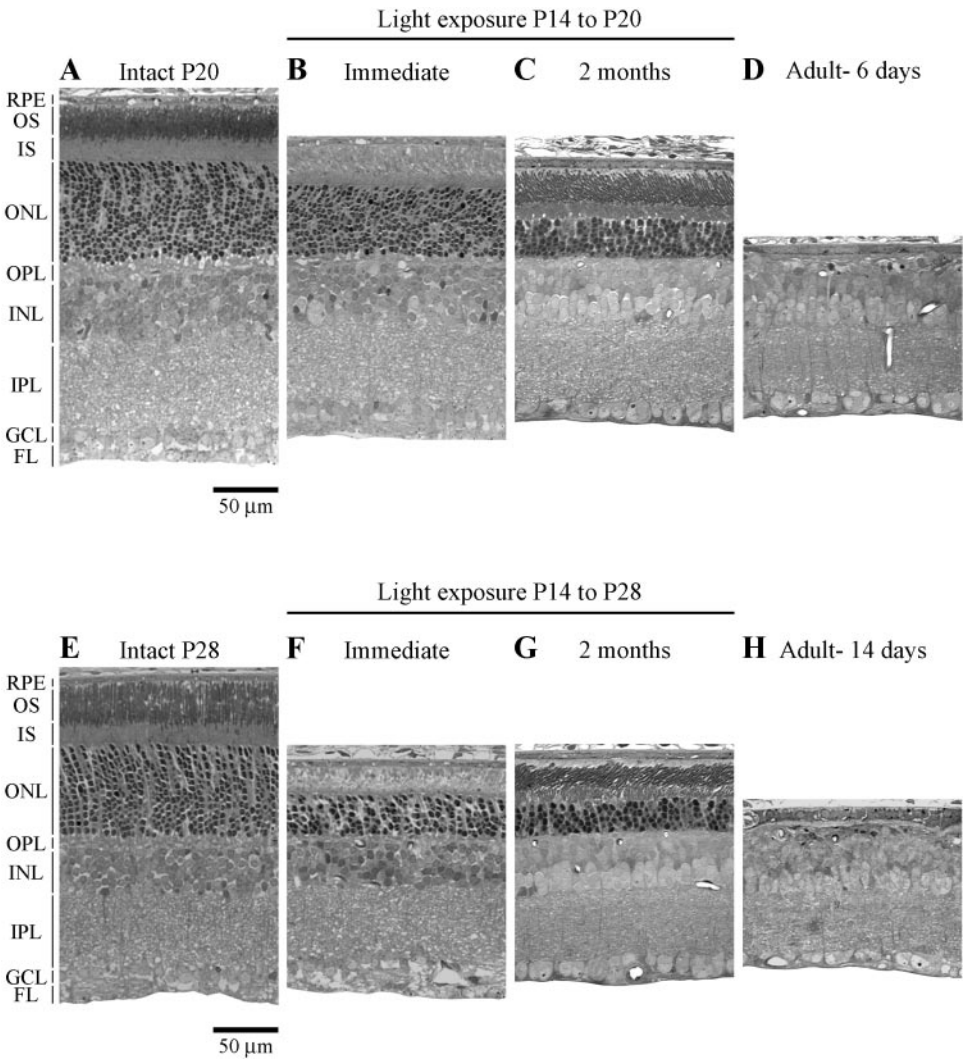


FIGURE 2. Representative photomicrographs of retinas obtained from juvenile rats exposed for 6 (P14–P20; **B**, **C**) and 14 (P14–P28; **F**, **G**) days to bright light. Retinas were collected immediately after 6 (**B**) or 14 (**F**) days of exposure and from control (unexposed) rats aged P20 (**A**) or P28 (**E**). Delayed damage was observed in 2-month-old retinas after 6 (**C**) and 14 (**G**) days of light treatment. Complete photoreceptor degeneration was observed in adult retinas 1 month after exposure for 6 (**D**) or 14 (**H**) days to the same intensity of light. Abbreviations are as in Figure 1. Scale bar, 50 μm.

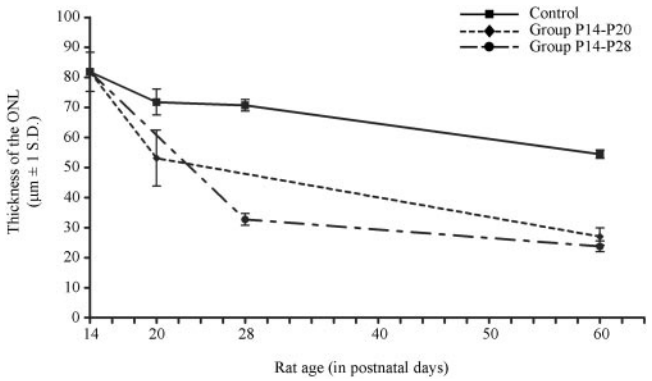


FIGURE 3. Modification of the ONL thickness with age (P14–P60) measured in control normal rats and in rats exposed to a bright environment for 6 (P14–P20) or 14 (P14–P28) consecutive days.

comparison, 14 days of exposure in adult rat led to a complete destruction of the external layers of the retina (Fig. 2H), confirming the relative resistance of the juvenile retina to light-induced degeneration. This resistance is best illustrated in Figure 3 where the thickness of the ONL is plotted against age in control (cyclic light) and light-damaged retinas. Despite minor differences in the ONL attrition process, both exposure regimens yielded equivalent ONL thickness (P14–P20: 27.0 ± 2.9 μm, $n = 3$; and P14–P28: 23.8 ± 1.8 μm, $n = 3$) at P60, which was approximately half the normal value ($P < 0.001$), independent of the duration of exposure to light.

The pathophysiological processes triggered by exposure to bright light are intimately tied to apoptosis.[10] For a better understanding of the cellular death process that occurs in our model of light-induced degeneration, we performed TUNEL staining in retinas from juvenile rats exposed for 6 (Fig. 4D) or 14 (Fig. 4E) consecutive days. Quantification of TUNEL-positive cells is shown in Figure 4F. A high number of cells were positive for TUNEL staining in the ONL after 6 or 14 days of exposure to light (Figs. 4D, 4E). TUNEL-positive cells were significantly more numerous after 6 days than after 14 days of exposure to light (Fig. 4F), whereas in the nonexposed rats, TUNEL staining was rarely observed (Figs. 4A–C, 4F).

In adult rats, mechanical retinal injury[33–35] and light-induced damage[22,36,37] lead to the upregulation of several neurotrophic factors. We hypothesized that an increase in endogenous levels of these factors may account for the intrinsic neuroprotection against light-induced damage observed in juvenile retinas. To test this theory, we examined the levels of retinal FGF-2 (Fig. 5A), CNTF (Fig. 5B), and BDNF (Fig. 5C) by Western blot analyses followed by densitometric analysis of the

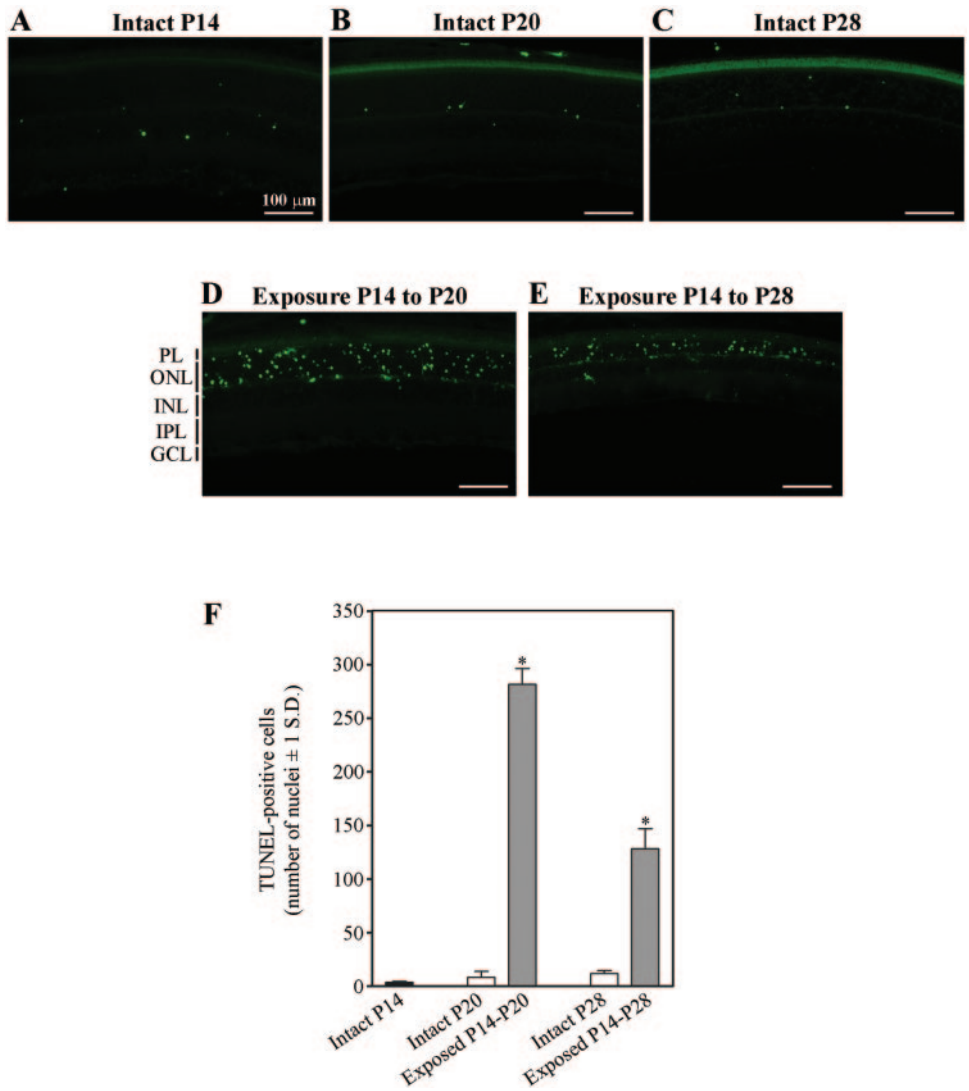


FIGURE 4. Representative examples of photoreceptor cell death as visualized with the TUNEL method. Retinal photomicrographs were obtained from normal (unexposed) rats aged P14 (**A**), P20 (**B**) and P28 (**C**) and from rats exposed for 6 (P14–P20; **D**) and 14 (P14–P28; **E**) consecutive days to bright light. Results are quantified in (**F**; ordinate: number of nuclei ± 1 SD). Statistical analysis was performed using a Student's *t*-test (*$P < 0.05$) comparing control animals (□) and exposed animals (■) after 6 and 14 days of exposure. Abbreviations are as in Figure 1. Scale bar, 100 μm.

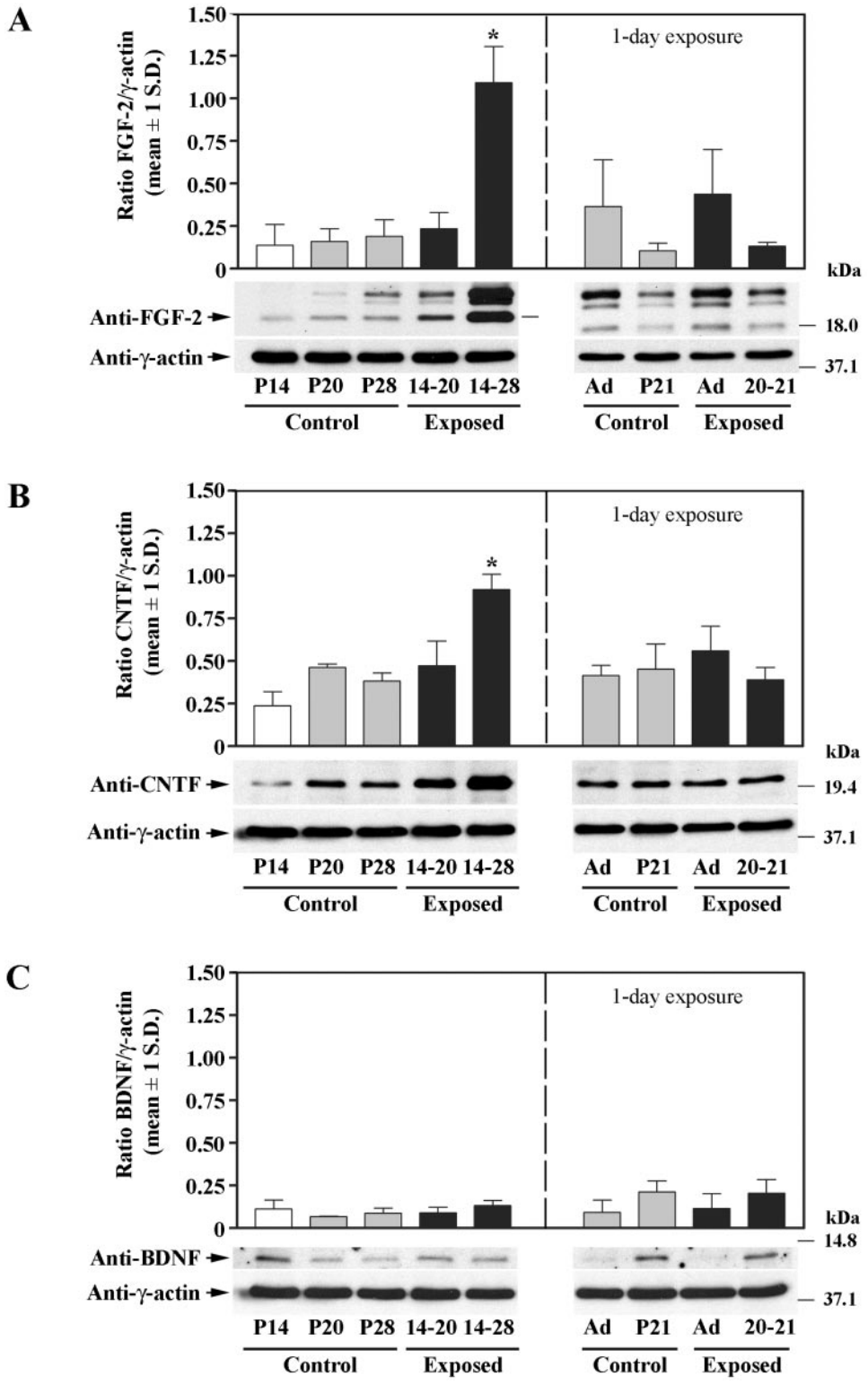


FIGURE 5. Variations of FGF-2 (**A**), CNTF (**B**), and BDNF (**C**) protein levels in exposed animals as measured by Western blot analysis. Retinas were collected from control (unexposed) rats at P14 (□), P20, and P28 (▩) and from rats exposed (■) for 6 days (P14–P20) or 14 days (P14–P28) to light. To determine the acute effect of light on neurotrophin expression, we collected retinas from control (nonexposed) adults (*lane Ad*; ▩) and from adults exposed for 1 day to light (*lane Ad*; ■). For comparison of adults and juveniles, retinas from juvenile rats at P21 were collected from nonexposed rats (▩) and rats exposed for 1 day (■). The quantification was performed by normalizing each neurotrophic factor level with that of the γ-actin (ordinate: ratio/γ-actin, mean ± 1 SD). *Right*: molecular mass (in kDa) of each band. For FGF-2, the signal is constituted by a doublet (22 and 24 kDa) and by a unique band at 18 kDa which was used for quantification. Statistical analysis was performed with a Student's *t*-test comparing animals exposed for 6 or 14 days (■) to age-matched control rats (▩).

bands on scanned autoradiograph film. Juvenile rat retinas were collected immediately after 6 and 14 days of exposure to light (groups P14–P20 and P14–P28) and from age-matched control rats (P14, P20, P28). After 14 days of exposure, FGF-2 protein levels (Fig. 5A) were upregulated by approximately fivefold compared with control retinas (P28 rats; $P < 0.01$, Student's *t*-test), whereas a 6-day exposure to light did not significantly change FGF-2 protein expression ($P > 0.05$). Similarly, a 14-day light regimen led to a significant increase in CNTF protein levels (~1.5-fold; $P < 0.001$, Student's *t*-test; Fig. 5B), while 6 days of exposure did not change the endogenous expression of CNTF protein. BDNF protein levels were not changed after either 6 or 14 days of exposure to light (Fig. 5C). To determine the acute effect of exposure to light on the expression of neurotrophic factors, adult rats were exposed for 1 day, since we had already demonstrated that this treatment was sufficient to inflict significant electrophysiological and morphologic damage on the retina.[17] Similarly, juvenile rats

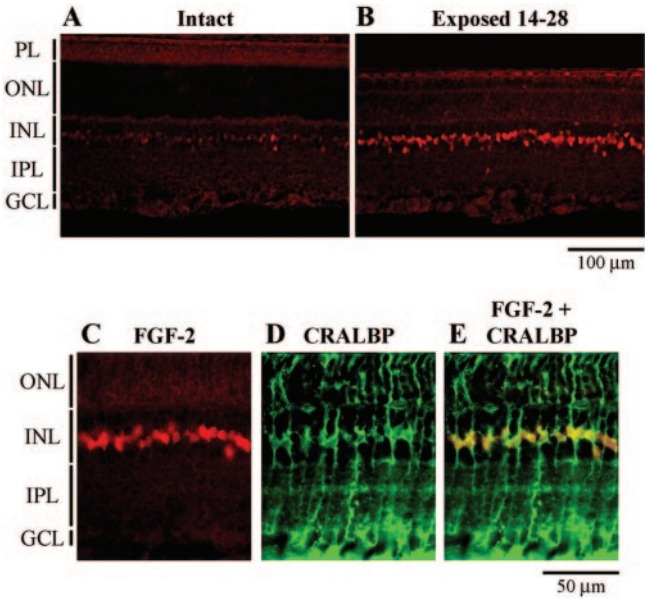


FIGURE 6. Cellular localization of FGF-2 in control (unexposed) retinas (**A**) and in retinas exposed for 14 days (**B**). Fluorescence microscopy of retinal sections revealed an upregulation of the FGF-2 staining in the inner nuclear layer (INL) (**B**). Colocalization of FGF-2 immunostaining (**C**) with anti-CRALBP staining (**D**), specific for cell bodies and radial processes of the Müller glial cells, demonstrated that Müller cells actively upregulated FGF-2 after exposure to light (**E**). Abbreviations are as in Figure 1. Scale bars: (**A**, **B**) 100 μm; (**C**–**E**) 50 μm.

were exposed to bright light for only 1 day (P20–P21). Our data demonstrate that a 1-day exposure was insufficient to yield a measurable increase in the levels of neurotrophic factors in adult as well as in juvenile rat retinas (Figs. 5A–C, right graphs).

To determine the cellular localization of endogenous FGF-2 and CNTF proteins after exposure to light, we performed retinal immunohistochemistry. Cyclic light treatment for 14 days led to strong FGF-2 staining in the inner nuclear layer (INL) compared with the basal levels in control retinas (Figs. 6A, 6B). The localization of FGF-2-positive cells in the INL suggested that these were Müller cells. To confirm the cell type, we used an antibody against cellular retinaldehyde binding protein (CRALBP), a Müller cell–specific marker. Figure 6 shows perfect colocalization of CRALBP and FGF-2 labeling in Müller cell bodies and radial processes, indicating that light-induced upregulation of FGF-2 occurred selectively in Müller glia (Figs. 6C–6E). Similarly, CNTF staining was observed in the INL of control animals (Figs. 7A, 7B) and was markedly upregulated after 6 or 14 days of exposure to bright light (Figs. 7C, 7D). CNTF colocalized with glial fibrillary acidic protein (GFAP), a marker for reactive Müller cells and astrocytes, demonstrating that Müller cell bodies and radial processes actively upregulated CNTF after exposure to light (Figs. 7E–G). These results identified the Müller glial cells as the primary source of high levels of endogenous FGF-2 and CNTF during light-induced damage.

During normal development, the thickness of the inferior and superior ONL remained basically identical (Fig. 8). Similarly, immediately after 6 or 14 consecutive days of exposure, the thickness of the ONL of both retinal hemispheres (inferior: Figs. 8C, 8I; superior: Figs. 8D, 8J) was similar (Fig. 8M; $P > 0.05$). However, at P60, the superior ONL was thinner ($P > 0.05$) than the inferior ONL after 6 days of exposure, a difference that reached a significant level after 14 days of exposure (Fig. 8; $P < 0.01$). Despite the obvious morphologic differences between the superior and inferior hemispheres after exposure to light, the levels of the neurotrophic factors FGF-2 and CNTF were identical in both retinal hemispheres in rats exposed to light for 6 days (P14–P20) and comparable to those measured in normal age-matched control rats (Fig. 8N), as revealed by Western blot analysis.

DISCUSSION

We have demonstrated that the retina of the juvenile rat displays a remarkable endogenous resistance to light-induced retinopathy compared with adult retinas,[17] a finding that was further confirmed with the results presented in this study. Of interest, the severity of the retinal degeneration after 6 (P14–P20) and 14 (P14–P28) days of exposure were not significantly different from each other when estimated at 2 months of age, despite significant differences in ONL thickness immediately after the end of exposure. Our data also reveal that after 14 days of light, the number of TUNEL-positive nuclei was reduced compared with 6 days of light, suggesting that light-induced photoreceptor death was almost complete immediately after 14 days of exposure to light. A possibility is that surviving photoreceptors have a built-in capacity to resist exogenous environmental stresses such as that used in the present study. This difference in response to an environmental stress might bear some similarities to that previously demon-

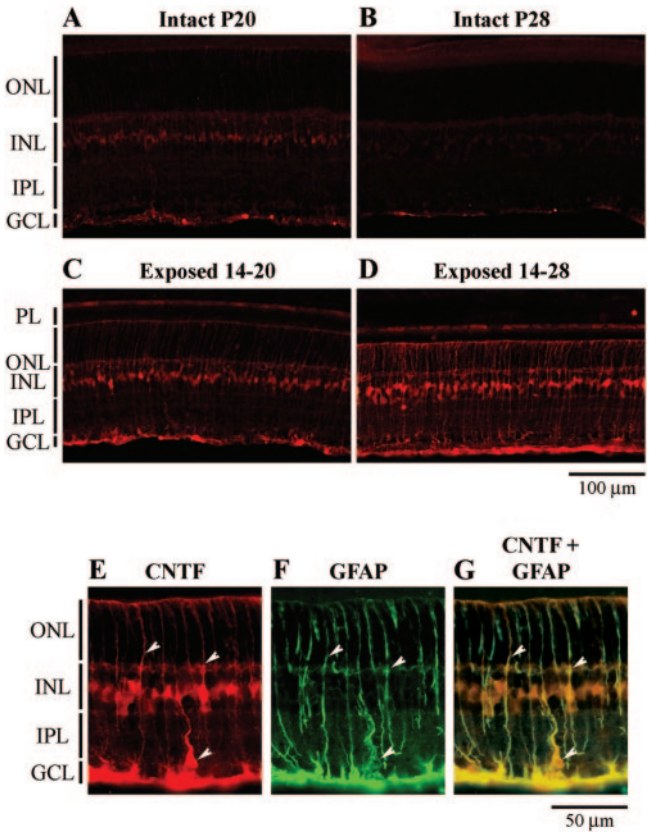
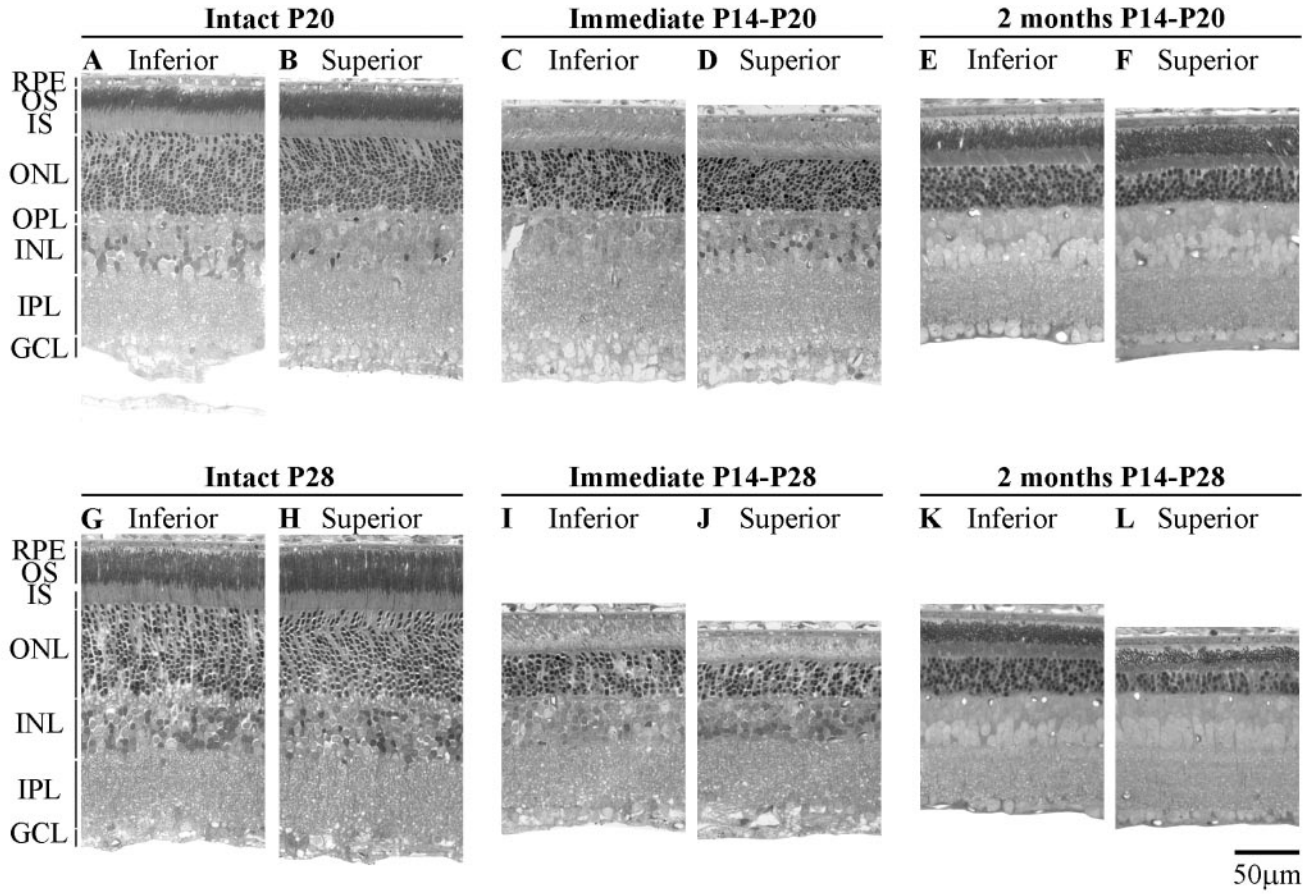
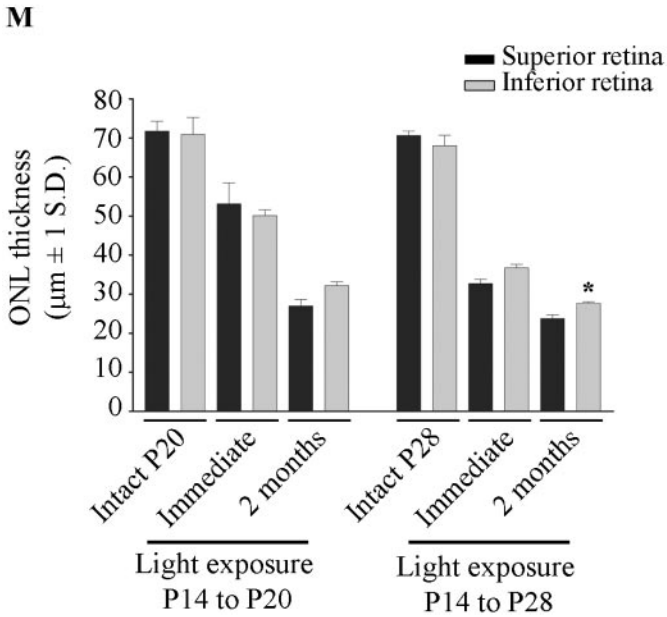


FIGURE 7. Cellular localization of CNTF in control (unexposed) retinas (**A**, **B**) and in retinas exposed to light for 6 (**C**) or 14 (**D**) days. Fluorescence microscopy of retinal sections revealed an upregulation of the CNTF staining in the inner nuclear layer (INL) after 6 (**C**) and 14 (**D**) days of exposure. Colocalization of CNTF immunostaining (**E**) with anti-GFAP labeling (**F**), specific for active Müller glial cells, demonstrated that Müller cells actively upregulated CNTF after exposure to light (**G**). Abbreviations are as in Figure 1. Scale bars: (**A-D**) 100 μm; (**E-G**) 50 μm.





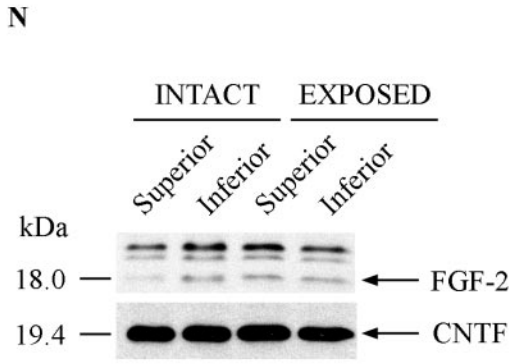


FIGURE 8. Representative photomicrographs showing the inferior (**A**, **C**, **E**, **G**, **I**, **K**) and superior (**B**, **D**, **F**, **H**, **J**, **L**) retinas of control (unexposed) rats at P20 (**A**, **B**) or P28 (**G**, **H**) and rats exposed to a bright light for 6 (P14–P20; **C**–**F**) or 14 (P14–P28; **I**–**L**) consecutive days. Retinas were collected either immediately after the cessation of exposure to light (**C**, **D**, **I**, **J**) or at 2 months of age (**E**, **F**, **K**, **L**). The thickness of the outer nuclear layer (ONL; in μm ± 1 SD) was quantified for each regimen of exposure in the superior and the inferior retinas (**M**). To determine changes in FGF-2 and CNTF protein levels in both retinal hemispheres, retinas were dissected into superior and inferior hemiretinal samples in normal rats and in juvenile rats exposed for 6 days (**N**). Left: molecular masses (kDa) of each Western blot. Abbreviations are as in Figure 1. Scale bar, 50 μm (**A**–**L**).

strated, to distinguish the inferior and superior retinal hemispheres in their reaction to exposure to bright light.[17]

Our data demonstrating that endogenous FGF-2 and CNTF are upregulated in Müller cells may underlie the relative protection of photoreceptors to light-induced damage that we observed in juvenile rats compared with adult animals. Previous studies have shown that in the adult retina, light-induced damage induces Müller cell gliosis[44] which, in turn, produces several neurotrophic factors.[23,35,36,39,45,46] Recently, Harada et al.[47] presented evidence suggesting that, during photodegeneration, activated microglia secrete factors that diffuse to the outer retina. These microglia-derived factors then trigger the production of trophic factors by Müller cells, leading to photoreceptor survival. Based on our results, we propose a similar mechanism whereby damaged photoreceptors may recruit microglial cells, for example via secretion of the monocyte chemoattractant protein-3 (MCP-3).[47,48] Activated microglia would then release neurotrophic factors such as GDNF that would, in turn, stimulate the secondary secretion of FGF-2 by Müller cells.[47] Neurotrophic factors may trigger photoreceptor survival by binding to their specific cognate receptors, including FGF receptor (FGFR)-1, the receptor for FGF-2 located in the ONL,[39,49,50] and CNTFRα, the CNTF receptor located in photoreceptor outer segments.[51,52]

Our results also demonstrate that endogenous levels of BDNF were not upregulated in the retina of juvenile rats in response to exposure to bright light. We have shown in adult albino rats[25] that BDNF gene transfer to Müller cells results in structural and functional protection of photoreceptors after constant exposure to light. In this study, BDNF-mediated neuroprotection was presumably due to the ability of adenovirus-infected Müller cells to secrete BDNF, which stimulated photoreceptor survival.[25] Alternatively, BDNF could exert a direct effect on Müller cells, as they express the BDNF receptor TrkB.[25,53] In the present study, however, we did not observe changes in endogenous BDNF levels that could support its role in the retinal response required to protect the developing retina from light-induced damage. This is consistent with other studies that have shown that BDNF mRNA levels, unlike FGF-2 or CNTF, were not changed after mechanical injury compared with those found during normal retinal development.[33,35]

In contrast to the results obtained after exposure to light for 14 days, a 6-day exposure was insufficient to alter the endogenous levels of FGF-2 and CNTF, raising the possibility that different retinal mechanisms are triggered to protect photoreceptors from light-induced damage. Taurine, an important amino acid found in very high concentrations in the vertebrate retina,[54] has been shown to participate in rod photoreceptor development by preserving the structure and organization of outer segments.[55,56] During normal retinal development, endogenous levels of taurine increase in mice[57] and rats,[58] especially between P15 and P21. Of note, exogenous administration of taurine in frogs can protect rod outer segments from light-induced damage in vitro and can inhibit light-induced lipid peroxidation.[55] Moreover, taurine has neuroprotective properties during development[59,60] and can promote regeneration of the central nervous system.[59] Its concentration increases drastically in the retina after optic nerve lesion[59] or after brain ischemia.[61] It is therefore possible that 6 days of exposure to light results in taurine upregulation above levels observed during normal maturation (P14–P20). This increase in taurine concentration may have protected photoreceptors from light-induced damage. Other studies have demonstrated that endogenous levels of antioxidant enzymes such as vitamin E, vitamin C and reduced glutathione were highly increased in rats reared in a bright environment compared with a dim environment.[12] Increased levels of these molecules may also have occurred in our model and could explain the protection that we observed after 6 days of exposure to light.

Other studies[9,17,41,62–64] have shown that, after exposure to bright light, the superior retina always developed a significantly more severe degeneration compared with the inferior retina, a finding that we were able to reproduce in the present study. Of note, whereas in the adult this hemiretinal difference could be demonstrated from analysis of retinal sections obtained immediately after cessation of the light-exposure regimen, a slightly different picture emerged when the results obtained from newborn rats were analyzed. As shown in Figure 8, whereas the typical hemiretinal difference is seen in retinas from 2-month-old rats, retinas from juvenile rats analyzed immediately after the cessation of exposure to light (at P20 or P28) did not reveal the expected difference between the superior and inferior retinal hemispheres. Although the reason behind the preservation of the superior retina in juvenile eyes remains to be established, contrary to our prediction, our results did not reveal that this process resulted from a significant increase in FGF-2 or CNTF protein levels in the superior retina compared with the inferior retina. This finding suggests that other mechanisms underlie the differential photoreceptor survival in retinal hemispheres. For example, it has been proposed that longer photoreceptor outer segments and, consequently, larger amounts of rhodopsin in the superior retina may generate higher amounts of free radicals and thus more damage after exposure to light.[41] In mice, the inferior retina displays a higher density of cones than the superior retina,[65] and cones are considered more resistant than rods to light-induced damage.[17,66–68] Together, these morphologic and biochemical properties of the rodent retina could explain why the superior retinal hemisphere is more sensitive to light-induced damage than the inferior hemisphere. They could not explain, however, the origin of the delayed response of the superior retina in establishing the well-documented hemiretinal difference.

In summary, our results provide evidence that FGF-2 and CNTF upregulation in Müller cells may participate in the mechanisms that protect photoreceptors from light-induced death. However, the relative contribution of these two neuroprotective agents remains to be determined. The use of function-blocking antibodies for FGF-2 and CNTF molecules may allow us to target the specific molecular pathways implicated in this juvenile resistance to light-induced damage.

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