Glucocorticoid-Induced Leucine Zipper Protects the Retina From Light-Induced Retinal Degeneration by Inducing Bcl-xL in Rats

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PURPOSE. The aim of the present study was to investigate the neuroprotective effects of glucocorticoid-induced leucine zipper (GILZ) in a light-induced retinal degeneration model and to explore the underlying mechanisms.

METHODS. Intravitreal injection of recombinant GILZ-overexpressing lentivirus (OE-GILZ-rLV) and short hairpin RNA targeting GILZ recombinant lentivirus (shRNA-GILZ-rLV) was performed to up- and downregulate retinal GILZ, respectively. Three days after stable transduction, rats were exposed to continuous bright light (5000 lux) for 2 days. Retinal function was assessed by full-field electrotoretinography (ERG), and the retinal structure was examined for photoreceptor survival and death in rats kept under a 12-hour light:2-hour dark cycle following light exposure. The expression levels of retinal Bcl-xL, caspase-9, and caspase-3 were examined by Western blotting or real-time PCR at 1, 3, 5, and 7 days after light exposure.

RESULTS. Exposure to bright light downregulated retinal GILZ in parallel with the downregulation of Bcl-xL and the upregulation of active caspase-3. Overexpression of retinal GILZ attenuated the decrease of Bcl-xL and the activation of caspase-9 and caspase-3 at 1, 3, 5, and 7 days after bright light exposure, respectively. GILZ silencing aggravated the downregulation of Bcl-xL induced by bright light exposure. Bright light exposure reduced the amplitude of ERG, increased the number of apoptotic photoreceptor cells, and decreased retinal thickness; and GILZ overexpression could attenuate all these effects.

CONCLUSIONS. Overexpression of GILZ by OE-GILZ-rLV transduction protected the retina from light-induced cellular damage by activating antiapoptotic pathways.

Keywords: apoptosis, Bcl-xL, GILZ, glucocorticoid-induced leucine zipper, light-induced retinal degeneration

Gluocorticoid-induced leucine zipper (GILZ), a member of the leucine zipper protein family, belongs to the transforming growth factor β-stimulated clone-22 family of transcription factors.1,2 It was first described as a glucocorticoid-induced protein in 1997 by D’Adamio et al.3 GILZ exerts anti-inflammatory effects by interacting with and inhibiting the key inflammatory signaling mediators nuclear factor-κB (NF-κB) and activator protein-1 (AP-1).4–8 In previous work, we found that GILZ could successfully inhibit inflammatory reactions in endotoxin-induced uveitis.9 GILZ inhibited the nuclear translocation of NF-κB p65 by promoting its dephosphorylation in rat primary retinal microvascular endothelial cells, leading to the inhibition of proinflammatory cytokine release.10 In addition to its anti-inflammatory effects, GILZ regulates numerous signal transduction pathways involved in cell proliferation and survival. Previously published data indicate that the cell survival regulatory functions of GILZ differ according to cell type. In 1997, D’Adamio et al.3 showed that GILZ could protect T cells from T cell receptor (TCR)/CD3-activated cell death, and Asselin-Labat et al.11 reported that GILZ delayed T-cell apoptosis by downregulating proapoptotic protein expression. In cancer cells, GILZ inhibits apoptosis by maintaining the levels of the prosurvival protein B-cell lymphoma-extra-large (Bcl-xL).12 In cardiomyocytes, GILZ overexpression protects cells from apoptosis by upregulating Bcl-xL expression, preventing cytochrome c release from mitochondria and caspase-3 cleavage.13 Bruscoli et al.14 used Gilz knockout mice to show that GILZ promotes B cell apoptosis. A similar phenomenon was reported in neutrophils, where GILZ overexpression exacerbated apoptosis in association with activation of caspase-3, caspase-9, and caspase-8, and the loss of mitochondrial potential.15 However, it is unclear whether GILZ regulates retinal neuronal survival or affects apoptosis in the retina.

Photoreceptor cell death and damage to the pigment epithelium often occur after exposure to excessive light.16,17 In animal models of light-induced retinal degeneration, the death of photoreceptor cells is predominantly caused by apoptosis.18 In the present study, a light-induced retinal injury model was used to investigate the neuroprotective effects of GILZ on the retina.

METHODS

Animals
All procedures were approved by the Animal Ethics Committee of the Eye and Ear Nose Throat Hospital of Fudan University,
China and were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Male Sprague-Dawley rats (approximately 200 g; 6–8 weeks old) were maintained in a 12-hour light:12-hour dark cycle with free access to food and water. A total of 61 rats were used in the present study. Rats were sacrificed by cervical dislocation under anesthesia induced by ketamine (80 mg/kg) and xylazine (10 mg/kg). All efforts were made to minimize suffering and reduce the number of rats used. The allocation of rats and treatments are summarized in Supplementary Table S1. For electroretinography (ERG) and retinal thickness measurements, both eyes in each rat were included. For other tests, only one eye in each rat was included.

Retinal Overexpression and Silencing of GILZ

GILZ overexpression and silencing were achieved using GILZ overexpressing recombinant lentivirus (OE-GILZ-rLV) and short-hairpin RNA targeting GILZ recombinant lentivirus (shRNA-GILZ-rLV), respectively (Genomeditech Co., Ltd., Shanghai, China). Two blank recombinant lentiviruses (blank-rLV) were used: the control virus for shRNA-GILZ-rLV and the control virus for OE-GILZ-rLV. To achieve stable transduction, 2 μL (1 × 10⁶ transducing units [TU]/mL) of the relevant lentivirus was intravitreally injected (Hamilton microinjector; Hamilton Bonaduz AG, Bonaduz, Switzerland) under a dissecting microscope. Three days after transduction, the rats were used in subsequent experiments.

Exposure to Bright Light

Three days after stable transduction, rats assigned to light exposure groups were exposed to continuously bright light to induce retinal degeneration in a light box. The animals were placed in individual boxes. The light source was provided by cool white fluorescent tubes. Fifteen tubes installed in the walls and roof of the light box delivered 5000 lux, as measured using a portable lux meter (LX1010B; Shanghai Handsun Electronic Co., Ltd., Shanghai, China) held 5 cm above the walls and roof of the light box delivered 5000 lux, as measured by passing them through a 25-gauge needle 10 times. The nuclear fraction and intact cells were removed by centrifugation at 300g for 10 minutes at 4°C. The supernatant was collected and centrifuged at 11,000g for 10 minutes at 4°C. The supernatant was collected as the cytosolic fraction, and the pellet was resuspended in mitochondrial isolation reagent B fractionation buffer. After being centrifuged at 12,000g for 10 minutes at 4°C, the pellet was collected as the mitochondrial fraction.

Western Blotting

The Sprague-Dawley rats were sacrificed at the specified times according to experimental design. After the anterior segment was removed, the neural retina was mixed with radioimmunoprecipitation assay buffer (Beyotime, Shanghai, China) and centrifuged at 4°C for 15 minutes and centrifuged at 12,000g for 15 minutes at 4°C. The supernatant was collected, and the protein concentration was measured using BCA protein assay (Beyotime). Then, 40 μg of protein from each sample was loaded and separated by SDS-PAGE and transferred to polyvinyl difluoride membranes (Millipore, Billerica, MA, USA). After membranes were blocked in 5% non-fat milk at room temperature for 1 hour, they were incubated with the following antibodies: rabbit anti-GILZ polyclonal antibody (1:500 dilution; ProteinTech, China), rabbit polyclonal caspase-3 antibody (1:500 dilution; product 19677-1-AP; Proteintech, China), rabbit polyclonal caspase-9 antibody (1:500 dilution; product 10880-1-AP; Proteintech), rabbit anti-p65 polyclonal antibody (1:500 dilution; Proteintech), and rabbit anti-β-actin antibody (1:1000 dilution; Abcam, Cambridge, MA, USA). Antibodies were diluted in 1% bovine serum albumin, and the blots were incubated at 4°C overnight. After being washed three times, the correct secondary antibodies were added, and the membranes were subjected to chemiluminescence detection (Pierce Biotechnology, Rockford, IL, USA). Chemiluminescent images were captured (Image Station, 4000MM PRO; Kodak, Rochester, NY, USA) and analyzed by using Image-Pro Plus software (ver. 6.0, Media Cybernetics, Bethesda, MD, USA). The band intensity was quantified and normalized relative to internal controls.

To separate cytosolic from mitochondrial fractions, a tissue mitochondrial isolation kit (Beyotime, China) was used. Briefly, fresh retina was collected and washed three times with phosphate-buffered saline (4°C). Then, 100 μL of mitochondria isolation reagent A (in 1 mM phenylmethane sulfonl fluoride) was added, and tissues were lysed by passing them through a 25-gauge needle 10 times. The nuclear fraction and intact cells were removed by centrifugation at 300g for 10 minutes at 4°C. The supernatant was collected and centrifuged at 11,000g for 10 minutes at 4°C. The supernatant was collected as the cytosolic fraction, and the pellet was resuspended in mitochondrial isolation reagent B fractionation buffer. After being centrifuged at 12,000g for 10 minutes at 4°C, the pellet was collected as the mitochondrial fraction.

Real-Time PCR

Total RNA was extracted from the retina by using Trizol RNA reagent (Invitrogen, Carlsbad, CA, USA). Each RT reaction included 0.5 μg of RNA, 2 μL of primer script buffer, 0.5 μL of oligo(dT), 0.5 μL of random hexamers, and 0.5 μL of primer script RT Enzyme Mix I (TaKaRa Bio, Inc., Shiga, Japan) in a total volume of 10 μL. Reactions were performed using a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA) for 15 minutes at 37°C, followed by heat inactivation for 5 seconds at 85°C. Then, 10 μL of the RT reaction mixture was diluted 10 times in nucleic-free water and stored at −20°C. Real-time PCR was performed using a LightCycler 480 II real-time PCR instrument (Roche, Basel, Switzerland) with 10 μL of PCR reaction mixture, which included 1 μL of cDNA, 5 μL of 2× LightCycler 480 SYBR Green I Master Mix (Roche), 0.2 μL of the forward primer, 0.2 μL of the reverse primer, and 3.6 μL of nucleic-free water. Reactions were performed in a 384-well optical plate (Roche) at 95°C for 10 minutes, followed by 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds. Each sample was run in triplicate. At the end of the PCR cycles, melting curve analysis was performed to validate the specific generation of the expected PCR product. The primer sequences were designed in the laboratory and synthesized by Generay Biotech (Shanghai, China) from the mRNA sequences published in the National Center for Biotechnology Information database; the primers are listed in Supplementary Table S2. The expression levels of each mRNA sample were normalized to those of Actb mRNA and calculated using the 2−ΔΔCt method.

Electroretinography

Blank-rLV was intravitreally injected into the right eye, and OE-GILZ-rLV was injected into the left eye. Three days after transduction, rats were exposed to 2 days of light injury. At 7 and 14 days after light exposure, the ERG was recorded. For the ERG, rats were dark-adapted for 2 hours and prepared for
recording under dim red illumination. Under dim red light illumination, rats were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Mydriasis was achieved by topical application of atropine sulfate. Oxybuprocaine (Santen Pharmaceutical Co., Ltd., Osaka, Japan) was applied topically for corneal anesthesia and Carbomer (Bausch & Lomb, Rochester, NY, USA) was applied for corneal hydration. The rat was supported on a warmed platform, and two contact lens electrodes were placed on both eyes to record full-field ERGs. A subcutaneous grounding electrode was placed on the tail. The reference electrode was affixed to the rat’s nose. Once the setup was complete, the dim red light was turned off, and rats were dark-adapted for 10 minutes before recording was started. The rod-ERG, max-ERG, cone-ERG, and flicker-ERG were recorded in order using the Espion visual electrophysiology system (Espion E3, Diagnosys UK Ltd., Cambridge, UK). The rod-ERG was recorded under 0 cd/s/m² background white light; the stimulus parameters were a single pulse flash of 0.01 cd/s/m², 0.1 Hz frequency, and 10 times superposition. The max-ERG was

**Figure 1.** Bright light exposure downregulated retinal GILZ expression. (A, B) The amount of GILZ was determined by Western blotting in the retina at 1, 3, 5, 7, and 14 days after 2 days of bright light exposure. (C, D) The amount of Bcl-xL was determined by Western blotting in the retina at 1, 3, 5, 7, and 14 days after 2 days of bright light exposure. (E, F) The amount of caspase-3 and active caspase-3 was determined by Western blotting in the retina at 1, 3, 5, 7, and 14 days after 2 days of bright light exposure. β-Actin was used as the loading control. Quantitative analysis of GILZ and Bcl-xL was performed by densitometry, and expression is shown relative to β-actin expression. The activation of caspase-3 is expressed as the active caspase-3-to-total caspase-3 ratio. Data mean ± SD; n = 3 per group. *P < 0.05 and **P < 0.01 (ANOVA and Bonferroni post-hoc test). NC, normal control.
recorded under 0 cd·s/m² background white light; the stimulus parameters were a single pulse flash of 20 cd·s/m², 0.1 Hz frequency, and 10 times superposition. After 5 minutes of photopic adaptation (50 cd·s/m²), the cone-ERG and flicker-ERG, respectively, were recorded. The cone-ERG was recorded under 50 cd·s/m² background white light; the stimulus parameters were a single pulse flash of 20 cd·s/m², 1 Hz frequency, and 20 times superposition; the flicker-ERG was recorded under 50 cd·s/m² background white light; the stimulus parameters were a continuous pulse flash of 20 cd·s/m², 10 Hz frequency, and 30 times superposition.

Histopathological Examination

After the ERGs were recorded, the rats were euthanized, and the eyes were immediately enucleated, fixed in 4% paraformaldehyde for 48 hours at room temperature, and kept in 70%
alcohol at 4°C until embedded in paraffin. Sagittal sections (5 μm) were cut near the optic nerve and stained with hematoxylin and eosin. Only one section was selected from each eye, and each group contained six eyes. Tissue was scanned using a digital slice scanning system (Panoramic Scan; 3DHistech, Budapest, Hungary), and the thicknesses of the total retina, outer nuclear layer (ONL), and inner nuclear layer (INL) were measured using Panoramic Viewer software (3DHistech Ltd.). The thickness of the retina was measured every 500 μm around the optic papilla on each retinal slice. The thicknesses of the total retina, ONL, and INL were measured in eight locations per sample.

TUNEL Staining

At 3 days after light exposure, the numbers of apoptotic neurons in blank-rLV– or OE-GILZ-rLV–transduced eyes were measured by TUNEL staining. The eyes were enucleated, and cornea and lenses were removed, and the eyecups were immersed in 4% paraformaldehyde for 2 hours. The eyecups were dehydrated in graded sucrose solutions (20%–30%) and embedded in OCT compound (Tissue-Tek; Ted Pella, Inc., Redding, CA, USA). The eyecups were then snap-frozen at −80°C and sectioned (10 μm) 20 minutes later. Air-dried sections were then treated with reagents from the In Situ Cell Death Detection Kit and fluorescein (Roche). Briefly, sections were washed three times in phosphate-buffered saline for 10 minutes and incubated for 60 minutes in 0.1% Triton X-100. Sections were washed three times in phosphate-buffered saline for 10 minutes and incubated for 60 minutes in 0.1% Triton X-100. Sections were immersed in TUNEL reaction mixture (50 μL of enzyme solution added to 450 μL of label solution) at 37°C for 60 minutes. After three rinses with 0.01 mol/L phosphate-buffered saline, the sections were counterstained with 4',6-diamidino-2-phenylindole (Sigma-Aldrich Corp. St. Louis, MO, USA) and examined using microscopy (Leica Microsystems, Bensheim, Germany). In each section, two areas 500 μm from the optic nerve head were selected for imaging. In each visual field, the number of TUNEL-positive cells was counted and averaged. Only one section was chosen from each eye, and each group contained three eyes.

Statistical Analysis

Statistical analyses were performed using SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA) for Windows (Microsoft, Redmond, WA, USA). The specific method is described in each figure legend.

RESULTS

Light Exposure Decreased the Retinal Expression of GILZ and Bcl-xL

As shown in Figures 1A and 1B, the retinal expression of GILZ was decreased at 1 day after bright light exposure, and this decrease persisted until day 14. The downregulation of GILZ in response to light damage occurred concomitantly with the downregulation of retinal Bcl-xL, an important antiapoptotic protein (Figs. 1C, 1D) and the activation of caspase-3 (Figs. 1E, 1F). These phenomena suggested that the downregulation of GILZ and Bcl-xL led to the activation of caspase-3.

GILZ Regulated the Expression of Retinal Bcl-xL and Rescued Mitochondria-Mediated Apoptosis

In response to apoptotic stimuli, Bcl-2-associated X (Bax) and Bcl-2 antagonist/killer 1 (Bak) (proteins of the B-cell lymphoma 2 family that can interact in the mitochondrial outer membrane to regulate its permeability) translocate to the mitochondrial membrane, facilitating the release of cytochrome c from the
mitochondrial intermembrane space into the cytosol.\textsuperscript{20–22} Cytosolic cytochrome c activates caspase-9 and caspase-3 and induces cell apoptosis.\textsuperscript{23} Bcl-xL inhibits the translocation of Bax/Bak and the release of cytochrome c, acting as an antiapoptotic protein.\textsuperscript{24} As shown in Figures 1C through 1F, the decrease in retinal Bcl-xL in response to bright light exposure was accompanied by the activation of caspase-3. To investigate the antiapoptotic function of exogenous GILZ in the retina, GILZ overexpression was induced by intravitreal injection of OE-GILZ-rLV (Figs. 2A, 2B). GILZ overexpression successfully attenuated the light exposure-induced downregulation of retinal Bcl-xL expression at the mRNA and protein levels (Figs. 2C–E). Consistent with the increase of Bcl-xL in GILZ-overexpressing eyes, the release of cytochrome c and activation of caspase-9 and caspase-3 were significantly decreased at 1, 3, 5, and 7 days after light injury compared with those in blank-rLV-transduced eyes (Figs. 3, 4). Silencing GILZ by intravitreal injection of short-hairpin RNA (shRNA)-GILZ-rLV (Figs. 5A, 5B) downregulated retinal Bcl-xL at the mRNA and protein levels at 1 day after light injury (Figs. 5C–E). These results indicate that exogenous GILZ protects retinal neurons from bright light damage by upregulating the expression of Bcl-xL and inhibiting the activation of mitochondrial apoptotic signaling pathways.

**GILZ Overexpression Protected Light Injury-Induced Photoreceptor Apoptosis**

TUNEL-positive nuclei were observed in the retina and were mostly located in the ONL at 3 days after light damage (Fig. 6A). Compared with blank-rLV, GILZ overexpression significantly decreased the number of TUNEL-positive nuclei (blank-rLV+C, 0.11 ± 0.33; blank-rLV+3 days after light exposure, 67.78 ± 10.94; and OE-GILZ-rLV+3 days after light exposure, 30.11 ± 7.72 per field; \( P < 0.01 \)) (Figs. 6A, 6B).

The thicknesses of the total retina, ONL, and INL were decreased at 7 and 14 days after light exposure in the blank-rLV- and OE-GILZ-rLV-transduced eyes compared with those in normal eyes. The thicknesses of the total retina and the ONL in GILZ-overexpressing eyes were much greater than those in blank-rLV-transduced eyes at 7 days after light injury. The differences between ONL thickness in the blank-rLV-transduced eyes and that in OE-GILZ-rLV-transduced eyes was observed at 14 days after light exposure (Fig. 7).

**GILZ Overexpression Attenuated the Loss of Retinal Function After Light Injury**

Electroretinography was used to assess retinal function, and the rod-ERG, max-ERG, cone-ERG, and flicker-ERG were recorded in
There were no differences in the ERG amplitudes (a-wave and b-wave amplitudes in rod-ERG, max-ERG, and cone-ERG, and the flicker-ERG amplitude) between blank-rLV- and OE-GILZ-rLV-transduced eyes in the normal group (Figs. 8, 9). The ERG amplitudes in blank-rLV- and OE-GILZ-rLV-transduced eyes at 7 days after light exposure were significantly lower than those in the control groups (Figs. 8, 9). GILZ overexpression significantly attenuated the decrease in the ERG amplitudes at 7 days after light exposure compared with those in blank-rLV-transduced eyes (Figs. 8, 9). The difference of a-wave in rod-and
max-ERG, b-wave in rod-ERG, and flicker-ERG between blank-rLV– and OE-GILZ-rLV–transduced eyes was still observed at 14 days after light exposure (Figs. 8, 9).

**DISCUSSION**

We used a light-induced model of retinal degeneration to demonstrate the neuroprotective effects of GILZ in the retina. GILZ overexpression attenuated light damage-induced photoreceptor apoptosis and rescued the loss of retinal function.

**GILZ downregulation is associated with inflammatory conditions.**

GILZ is downregulated in the lipopolysaccharide-stimulated lung, in degenerated venous bypass, in activated macrophages from patients with Crohn’s disease, and in the livers of patients with alcoholic hepatitis. Our previous research showed that GILZ was downregulated in endotoxin-induced uveitis and in the aqueous humor of
GILZ overexpression rescued the loss of retinal neurons caused by bright light exposure. (A–E) Representative photomicrographs show the histological appearance of retinas from the control and light-damaged groups. (A) Blank-rLV+ C; (B) blank-rLV+ 7 days after light exposure; (C) OE-GILZ-rLV+ 7 days after light exposure; (D) blank-rLV+ 14 days after light exposure; and (E) OE-GILZ-rLV+ 14 days after light exposure. Images were taken 500 μm from the optic papilla. (F–H) Quantitative analysis of the retinal thicknesses: (F) total retinal thickness (from the RPE to the ILM); (G) INL; and (H) ONL. Data are mean ± SD. n = 6 per group. *P < 0.05 and **P < 0.01 (Mann-Whitney U test). C, control; ILM, internal limiting membrane; OE, overexpressing; rLV, recombinant lentivirus; RPE, retinal pigment epithelium.

bacterial endophthalmitis. At 1 day after bright light exposure, the expression of retinal GILZ was decreased, and this decrease persisted for up to 14 days. These results indicate that downregulation of GILZ is a general feature of inflammation and that it occurs in association with other pathological processes, such as light-induced photoreceptor degeneration.

Proteins of the Bcl-2 family interact in the mitochondrial outer membrane to regulate its permeability. These proteins contain one or more Bcl-2 homology (BH) motifs and either promote or inhibit mitochondrial outer membrane permeabilization, which initiates or inhibits apoptosis. Bax and Bak contain three BH motifs (BH1–3) that are required for mitochondrial outer membrane permeabilization. In response to apoptotic stimuli, Bax/Bak translocate to the mitochondrial membrane, facilitating the release of cytochrome c from the mitochondrial intermembrane space into the cytosol. Cytosolic cytochrome c activates caspase-9 and caspase-3 and, hence, cell apoptosis. Bcl-xL, an antiapoptotic protein, contains four BH motifs (BH1–4) and functions as a direct and indirect inhibitor of Bax and Bak.
the activation of caspase-9 and caspase-3 in the retina in response to light injury. In OE-GILZ-rLV–transduced eyes, GILZ overexpression upregulated retinal Bcl-xL and downregulated the activation of these apoptotic signaling pathways at 1, 3, 5, and 7 days after light exposure compared with blank-rLV. The relationship between GILZ and Bcl-xL was reported previously, and the antiapoptotic function of GILZ is mediated by the upregulation of Bcl-xL. In the present study, GILZ overexpression upregulated retinal Bcl-xL, whereas GILZ silencing enhanced the decrease in retinal Bcl-xL at the mRNA and protein levels. These results indicate that the antiapoptotic effects of GILZ in the retina may be mediated by the maintenance of retinal Bcl-xL levels.

The mechanism underlying the regulation of Bcl-xL by GILZ in the retina remains unclear. In the present study, we showed that GILZ regulated Bcl-xL at the protein and mRNA levels, suggesting that GILZ regulates Bcl-xL expression at the transcriptional level. However, previous studies have suggested that GILZ acts as a transcriptional repressor by interacting with nuclear transcription factors and inhibiting transcriptional activity. At 1 day after bright light damage, retinal NF-kB p65 had translocated from the cytoplasm to the nucleus in blank-rLV–transduced eyes, but this translocation was attenuated in OE-GILZ-rLV–transduced eyes (Supplementary Fig. S1). This indicates that GILZ acts as a transcriptional repressor in this light-induced model of retinal degeneration. Therefore, we hypothesized that GILZ might promote Bcl-xL transcription through an indirect pathway. As shown in Supplementary Figure S2, GILZ overexpression significantly downregulated retinal tumor necrosis factor-α (TNF-α) expression in the light-induced retinal injury model compared with blank-rLV. Previous studies showed that TNF-α downregulates Bcl-xL in various cell types. Therefore, we hypothesized that GILZ inhibits proapoptotic factors such as TNF-α by inhibiting NF-κB p65 transcriptional activity, and the downregulation of proapoptotic factors allows upregulation of Bcl-xL in the retina. TUNEL staining showed that most of the apoptotic cells following light damage were photoreceptor cells and that GILZ overexpression significantly protected the apoptosis of photoreceptors. However, retinal transduction was induced by intravitreal injection of lentivirus and any retinal cells could be transduced. This indicates that inhibition of retinal TNF-α may occur in photoreceptor cells or any other retinal cells after light injury in OE-GILZ-rLV–transduced eyes. Therefore, GILZ overexpression downregulates retinal TNF-α (TNF-α may be produced by photoreceptor cells or other retinal cells), which results in the maintenance of Bcl-xL levels and the inhibition of photoreceptor cell apoptosis. Viral vectors that can specifically transduce photoreceptor cells should be designed in the future to verify whether the downregulation of TNF-α is mediated by retinal photoreceptor cells in the light-induced model of photoreceptor degeneration.

Electroretinography is a noninvasive and objective measurement of retinal function and is widely used for the diagnosis of visual disorders. Several studies demonstrated that exposure of the rat retina to light leads to significant reductions
FIGURE 9. Quantitative analysis of ERG traces. (A) A-wave amplitudes of rod-ERG. (B) A-wave amplitudes of max-ERG. (C) A-wave amplitudes of cone-ERG. (D) B-wave amplitudes of rod-ERG. (E) B-wave amplitudes of max-ERG. (F) B-wave amplitudes of cone-ERG. (G) Flicker-ERG amplitudes. Data are mean ± SD. n = 6 per group. *P < 0.05 and **P < 0.01 (Mann-Whitney U test).
in ERG amplitudes.\textsuperscript{38–41} The ERG a-wave originates from photoreceptor cells, and the b-wave originates from bipolar cells.\textsuperscript{42,43} The retinal origin of the flicker-ERGs has not been confirmed, although they might originate from the cone system.\textsuperscript{44–46} In the present study, the rod-ERG, max-ERG, cone-ERG, and flicker-ERG were recorded in order, and the amplitudes of these ERG parameters were significantly decreased at 7 and 14 days after light injury compared with those in the control groups. GILZ overexpression significantly attenuated the reductions in ERG amplitudes, suggesting that retinal neurons were damaged by light in this model of photoreceptor degeneration and that GILZ overexpression protects retinal neurons from light damage.

The present study had some limitations. The overexpression of GILZ was induced by intravitreal injection of a lentivirus, which might have transduced photoreceptor cells and other retinal cells. Therefore, it is unclear whether the effects of GILZ overexpression were mediated directly by photoreceptor cells or indirectly by other retinal cells. Viral vectors capable of specifically transducing photoreceptor cells should be designed in the future.

**Conclusions**

The present study used a light-induced model of retinal degeneration to analyze the retinal effects of GILZ. GILZ overexpression attenuated photoreceptor cell apoptosis, the activation of apoptotic signaling pathways, ERG amplitudes, and changes in retinal thickness associated with light damage, indicating that GILZ has neuroprotective effects in the retina.

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