Sustained Ocular Hypertension Induces Dendritic Degeneration of Mouse Retinal Ganglion Cells That Depends on Cell Type and Location

Liang Feng,^{1,2} Yan Zhao,³ Miho Yoshida,² Hui Chen,¹ Jessica F. Yang,² Ted S. Kim,² Jianhua Cang,² John B. Troy,³ and Xiaorong Liu^{1,2}

PURPOSE. Glaucoma is characterized by retinal ganglion cell (RGC) death and frequently associated with elevated IOP. How RGCs degenerate before death is little understood, so we sought to investigate RGC degeneration in a mouse model of ocular hypertension.

METHODS. A laser-induced mouse model of chronic ocular hypertension mimicked human high-tension glaucoma. Immunohistochemistry was used to characterize overall RGC loss and an optomotor behavioral test to measure corresponding changes in visual capacity. Changes in RGC functional properties were characterized by a large-scale multielectrode array (MEA). The transgenic Thy-1-YFP mouse line, in which a small number of RGCs are labeled with yellow fluorescent protein (YFP), permitted investigation of whether subtypes of RGCs or RGCs from particular retinal areas were differentially vulnerable to elevated IOP.

RESULTS. Sustained IOP elevation in mice was achieved by laser photocoagulation. We confirmed RGC loss and decreased visual acuity in ocular hypertensive mice. Furthermore, these mice had fewer visually responsive cells with smaller receptive field sizes compared to controls. We demonstrated that RGC dendritic shrinkage started from the vertical axis of hypertensive eyes and that mono-laminated ON cells were more susceptible to IOP elevation than bi-laminated ON-OFF cells. Moreover, a subgroup of ON RGCs labeled by the SMI-32 antibody exhibited significant dendritic atrophy in the superior quadrant of the hypertensive eyes.

CONCLUSIONS. RGC degeneration depends on subtype and location in hypertensive eyes. This study introduces a valuable model to investigate how the structural and functional

degeneration of RGCs leads to visual impairments. (*Invest Ophthalmol Vis Sci.* 2013;54:1106–1117) DOI:10.1167/iov.12-10791

Glaucoma is a leading cause of blindness, characterized by retinal ganglion cell (RGC) death.^{1,2} Different animal models of experimental glaucoma have been established to mimic features of glaucomatous optic neuropathy.³ Because elevated IOP is a major risk factor for the development and progression of glaucoma, acute or chronic ocular hypertension is often induced in mouse eyes to mimic human high-tension glaucoma.^{4–6} Subsequent IOP elevation and RGC loss have been confirmed.^{7,8} In this article, we report how, in a mouse model of sustained ocular hypertension, the progression in RGC degeneration proceeds toward cell death and corresponding loss of visual performance.

There are many subtypes of RGCs, each with a unique dendritic morphology and function in vision.^{9–13} Diversity in RGC damage has been reported in glaucomatous retinas^{8,14}; however, studies in human patients and animal models have so far failed to provide a clear picture of whether RGC degeneration and subsequent death depends on cell type and/or location. Some work suggests a selective loss of subtype RGCs in glaucoma^{15–17}; but, although RGCs with small cell bodies dominate in the glaucoma model of DBA2/NNia mice,¹⁸ this could indicate either a preferential loss of larger RGCs or a general reduction in cell size. Other studies have shown that RGC death also depends on retinal location.^{19–21} Studies of dendritic degeneration in glaucoma suggest that there may be some dependence on cell type,^{7,22–24} but this remains an area in need of fuller investigation. This is particularly so because, although abnormalities in RGC structure probably accompany visual impairment, no one has yet demonstrated that changes in RGC morphology occur in parallel with changes in visual performance in glaucoma. Some glaucoma patients have a lower amplitude pattern electroretinogram, a signal mainly generated by RGCs.^{25,26} In the glaucomatous mouse²⁷ and monkey,^{24,28,29} RGCs are also found to be less responsive to visual stimulation. But we do not know if these effects result from changes in RGC dendritic structure with the resulting loss of synaptic drive or because retinal neurons presynaptic to RGCs are themselves lost.

Given that dendritic structure determines an RGC's function in visual information processing⁹ and that damage in RGC dendrites has been observed in glaucomatous retinas,^{8,14} a comprehensive assessment of RGC dendritic degeneration is critical for us to better understand the progressive vision loss in glaucoma. Moreover, glaucoma is a “silent” disease that progresses without obvious symptoms. It is therefore often diagnosed too late for effective treatment. Because the deterioration of dendritic morphology precedes RGC death

From the Departments of ¹Ophthalmology, ²Neurobiology, and ³Biomedical Engineering, Northwestern University, Evanston, Illinois.

Supported by the Dr Douglas H. Johnson Award for Glaucoma Research from the American Health Assistance Foundation (XL), the William & Mary Greve Special Scholar Award from Research to Prevent Blindness (XL), the Illinois Society for the Prevention of Blindness (LF, HC), National Science Foundation Grant DBI-0551852 (JBT), and National Institutes of Health Grants R21EB004200 (JBT), R01EY018621 (JC), and R01EY019034 (XL).

Submitted for publication August 17, 2012; revised November 18, 2012; accepted December 26, 2012.

Disclosure: L. Feng, None; Y. Zhao, None; M. Yoshida, None; H. Chen, None; J.F. Yang, None; T.S. Kim, None; J. Cang, None; J.B. Troy, None; X. Liu, None

Corresponding author: Xiaorong Liu, Departments of Ophthalmology and Neurobiology, Northwestern University, 205 Tech Drive, Hogan 2-160, Evanston, IL 60208; xiaorong-liu@northwestern.edu.

in glaucomatous eyes,^{8,14,30} understanding how an RGC degenerates before it dies is important for detection and therapeutic treatment of glaucoma. In this study, we characterized the subtype- and location-dependent dendritic degeneration of RGCs in a mouse model of laser-induced chronic ocular hypertension.

MATERIALS AND METHODS

C57BL/6 wild-type (WT) and Thy-1-YFP transgenic mice with C57BL/6 background (Line H; The Jackson Laboratory, Bar Harbor, ME) reared in 12 hours of light/12 hours of darkness were used for this study. All animal procedures were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the guidelines on the Use of Animals in Neuroscience Research from the National Institutes of Health, and were approved by the Northwestern University Institutional Animal Care and Use Committee.

Laser Photocoagulation and IOP Measurements

We modified the procedure of laser photocoagulation described previously.^{4,5,31} In detail, 40- to 60-day-old mice were anesthetized by an intraperitoneal injection of ketamine (100 mg/kg; Butler Schein Animal Health, Dublin, OH) and xylazine (10 mg/kg, Lloyd, Inc., of Iowa, Shenandoah, IA). The pupil of the right eye of the experimental animal was dilated by topical treatment with one or two drops of 1% atropine sulfate solution (Alcon Labs, Inc., Fort Worth, TX). After mydriasis, the anterior chamber was flattened to enhance laser induction.⁴ The right eye of the anesthetized mouse was then aligned under the slit lamp (SL-3E; Topcon, Oakland, NJ) and illumination applied to its corneal limbus using an Argon laser (Ultima 2000SE; Coherent, Santa Clara, CA). Approximately 80 to 100 laser spots (514 nm, 100 mW, 50 ms pulse, and 200 μ m diameter) were delivered perpendicularly around the circumference of the trabecular meshwork to induce synechial angle closure. Topical 0.5% moxifloxacin (Alcon Labs, Inc.) and 0.5% proparacaine (Bausch & Lomb, Rochester, NY) were instilled on the ocular surface to disinfect the lasered area and to relieve pain. The untreated left eye served as a control.

The IOP was measured for all treated eyes during the whole time period. Three consecutive sets of six measurements of IOP were acquired using a rebound tonometer (TonoLab; Colonial Medical Supply, Franconia, NH).³² The normal IOP of most mice falls into the range of 12 to 17 mm Hg (untreated eyes).³³ Compared with baseline IOP, all the laser-treated eyes had at least a 50% increase of IOP within 1 month after treatment.

Optomotor Test

The eyes of lasered mice were examined by naked eyes of observers and under a dissecting microscope to identify if they had cataracts, corneal scars, or other physical damage. Approximately 20% to 30% of all lasered mice exhibited no ocular abnormality, inflammation, or other physical signs of damage to the eyes. These mice formed the sample of experimental subjects tested for visual behaviors.

Visual acuity and contrast sensitivity were tested.^{32,34} The two eyes of individual mice were examined separately by reversing the drifting grating direction (i.e., a clockwise drifting grating was used to identify the visual function of the left eye, and a counterclockwise drifting grating for the right eye).³⁵ The contrast threshold for each eye was measured separately at two preselected spatial frequencies: 0.075 and 0.161 cycle per degree (cpd). The contrast sensitivity is the reciprocal of the threshold.³⁶

Immunohistochemistry

Sectioned and whole-mount retinas were prepared for immunostaining as described previously.¹⁰ For whole-mount samples, retinas were

nicked on the nasal side during dissection. The primary antibodies used in this study included rabbit anti-GFP (1:1000; Invitrogen A-6455, Carlsbad, CA), mouse anti-Brn3a (1:400; Millipore MAB1585, Billerica, MA), goat anti-Brn3b (1:1000; Santa Cruz Biotechnology sc-6026, Santa Cruz, CA), mouse anti-tyrosine hydroxylase (TH; 1:400, Millipore MAB318), and mouse anti-SMI-32 (1:1000; Covance, Princeton, NJ). Alexa Fluor-conjugated secondary antibodies were used (1:1000; Invitrogen) to visualize the staining. After immunostaining, images were captured with a Zeiss Pascal confocal microscope (Zeiss, Thornwood, NY). For cell counting, 16 to 20 micrographs per retina were used to quantify the cell numbers in Imaris (Bitplane AG, Zürich, Switzerland). Retinal sections stained by 4'-6-diamidino-2-phenylindole were examined by a Zeiss ObserverA1 microscope.³²

For whole-mount samples, retinas were flat-mounted on the slides like a clover leaf with 4 quadrants: superior, inferior, nasal, and temporal. RGCs were classified into ON, OFF, and ON-OFF subtypes based on their laminar pattern of dendritic stratification.¹⁰ Z-stack images of YFP-expressing RGCs were projected onto a single two-dimensional plane and the field size of RGC arbors was measured using the polygon tool to link the outermost dendritic tips in ImageJ.^{11,37} The RGC dendritic length and the number of branches were measured using the Imaris 3D tracing program (Bitplane AG).¹⁰

Axon Counting

Optic nerves were fixed in glutaraldehyde in EOPN-Araldite resin (Electron Microscopy Sciences, Hatfield, PA) and cut into 1- μ m sections. The sections were then stained with p-phenylenediamine (Sigma-Aldrich, St. Louis, MO) and imaged under a Zeiss inverted microscope. A series of 20 images for each optic nerve were taken in the following positions: center, mid-periphery, and peripheral margin to cover different areas of the optic nerve. The axon numbers per image were counted manually using OPTIMAS 6 software (Optimas, Bothell, WA), averaged, and then normalized to the total area of the optic nerve. The axon numbers in laser-treated hypertensive eyes were compared with the untreated controls and the axonal loss expressed as the percentage difference.

Multielectrode Array Recordings

We laser-treated the right eyes of 1.5-month-old mice and then examined the visual responses of RGCs at 1.5 months after laser treatment (i.e., 3-month-old mice). Mice were dark adapted for 1 to 2 hours before the experiments.³⁸ Control and ocular hypertensive mouse retinas were dissected, and placed into contact with a 256-channel multielectrode array (MEA) (MEA-200/30iR-ITO; Multichannel Systems GmbH, Reutlingen, Germany).³⁸ During the recording session, the retina was super-perfused with oxygenated (95% O₂, 5% CO₂) artificial cerebrospinal fluid at 33.5°C.^{38,39} Voltage signals from the MEA were sampled at 25 kHz and the spike trains detected using a 5.5-SD voltage threshold in MCRack (Multichannel Systems GmbH), sorted into units in Offline Sorter (Plexon, Dallas, TX), and exported to MATLAB (Mathworks, Natick, MA) for further analysis.³⁸

A pattern of spatiotemporal Gaussian white noise was generated on an LCD display (KCD-VDCF-BA; Kopin, Taunton, MA) and projected onto the retina to characterize the visual responses of RGCs.³⁸ The Gaussian white noise had a mean luminance of 2 cd·m⁻² and appeared as a flickering gray-scale checkerboard composed of 100 $\times$ 100- μ m square checkers with random spatial and temporal properties.³⁸

The visual responses of RGCs to the Gaussian white noise were analyzed through spike-triggered average (STA) analysis.³⁸ We calculated the STA by averaging all stimulus frames in a 1-second window that preceded a spike, generating a video of the mean effective stimulus for triggering a spike. The STA provides an estimate of the linear spatiotemporal receptive field (RF) properties of RGCs.⁴⁰ The spatial extent of the RF center is modeled as a two-dimensional Gaussian,⁴¹ with magnitude normalized using the STA's mean luminance and its SD. We classified cells with an RF center magnitude greater than 4 (i.e., 4

FIGURE 1. Laser photocoagulation of the aqueous humor outflow in mouse eyes. **(A)** Schematic diagram of the laser treatment. **(B)** Sections of a control eye with open angle and a laser-treated eye with angle-closure. Stars indicate the areas that were expanded below. Scale bars: 200 μm . **(C)** IOP increases after laser treatment. IOP was measured at 1, 3, and 7 days, and then weekly for 26 weeks after laser treatment. 0 to 4 weeks: $n = 77$ mice; 5 to 8 weeks: $n = 51$; 9 to 12 weeks: $n = 29$; 13 to 16 weeks: $n = 14$; 17 to 26 weeks: $n = 11$. Error bar: SEM, same throughout.

FIGURE 2. Ganglion cell density is decreased following laser treatment. **(A)** Whole-mounted retinas immunostained by Brn-3a antibody. Scale bar: 500 μm . **(B)** Cell density of Brn-3a (left) and Brn-3b (right) from laser-treated (colored bars) and untreated eyes (black bars) at 1, 2, and 6 months after the laser treatment. ** $P < 0.01$; *** $P < 0.005$ in the Student's t -test. **(C)** Axon loss after laser treatment. Left: Sections to show stained axons in untreated and treated optic nerves. Scale bar: 10 μm . Right: Percentage axon loss at 1 to 12 weeks after laser treatment. $n = 5$ to 6 mice in each group. **(D)** Retinal sections from laser-treated and untreated eyes immunostained by PKC α and calretinin antibodies show that the overall morphology of the inner retina was unaffected by IOP elevation. Scale bar: 50 μm .

FIGURE 3. Decrease of visual acuity and contrast sensitivity with IOP elevation. (A) Photos of laser-treated eye and control eye. (B) Visual acuities were measured before and at 2 months and 5 to 6 months after laser treatment. (C) Contrast sensitivity at 2 months after laser treatment. ** $P < 0.01$; and *** $P < 0.001$ in Student's *t*-test.

SDs above or below the mean) as visually responsive. An RGC was deemed nonresponsive if no element of its STA exceeded 4 SDs. On visual inspection, we confirmed that the STAs of cells below this threshold lacked a defined RF.

RF center areas were computed from the STA, in which the single frame possessing the maximal deviation from the mean was isolated, and a bivariate Gaussian distribution was fit to this frame.³⁸ The RF center area was calculated within the 1 SD (σ) ellipse of the Gaussian distribution.³⁸

Statistical Analysis

Results were expressed as the mean $\pm$ SE (SEM). The Student's *t*-test was performed to compare paired samples, the Kolmogorov-Smirnov (K-S) test to compare the distributions of two samples, and one-way ANOVA to compare multiple samples in a group.

RESULTS

Sustained Ocular Hypertension Is Induced by Laser Photocoagulation in Mice

Laser illumination was applied to the corneal limbus of the right eye (Treated) of the target animal, whereas the left eye served as an internal control (Untreated; Fig. 1, see details in Materials and Methods). As shown in Figure 1B, laser photocoagulation induced angle closure, whereas the overall structure of the rest of the parts of the eye was largely normal. We further examined the retinas at 1 hour and 1 day after laser treatment and found no difference between control and lasered retinas (see Supplementary Material and Supplementary Fig. S1, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10791/-/DCSupplemental>), supporting the belief that laser illumination itself did not damage the retina.

Before laser treatment, the IOP baselines of the two eyes of all mice fell into the normal range³⁵ and showed no difference: 14.8 ± 0.1 (right) versus 14.9 ± 0.1 mm Hg (left, day 0, $n = 77$ mice, Fig. 1C). Three days after laser treatment, the mean IOP of the treated (right) eyes increased almost 2-fold to 28.6 ± 0.6 mm Hg, compared to the untreated eyes (15.1 ± 0.1 mm Hg, $P < 0.001$ in Student's *t*-test, same below). The IOP of laser-treated eyes remained elevated at 26 to 28 mm Hg for approximately 4 months (Fig. 1C). At 4 months after treatment, the mean IOP of treated eyes was 23.4 ± 1.9 mm Hg, significantly higher than that of untreated (left) eyes (15.3 ± 0.3 mm Hg, $n = 14$, $P = 0.015$). Subsequently, the mean IOP of treated eyes slowly decreased and reached 20.8 ± 1.3 mm Hg at 6 months (24 weeks) after treatment, but still higher than that of untreated eyes (15.5 ± 0.1 mm Hg, $n = 11$, $P = 0.025$). Together, our data demonstrated that IOP elevation was achieved by laser photocoagulation in mice and this elevation

was sustained for months. We next examined whether RGC loss occurred during these months.

Ganglion Cell Density Decreases After Laser Treatment

We confirmed a gradual decrease in RGC density after laser treatment using four methods (Fig. 2, Table). First, retinas were immunolabeled with two markers for RGC subtypes, Brn-3a (Fig. 2A) and Brn-3b antibodies, at 1, 2, and 6 months after laser treatment and compared with untreated eyes of the same target animals (Fig. 2B). We observed 9.1%, 16.1%, and 19.7% reductions in Brn-3a cell density at 1, 2, and 6 months after laser treatment (Fig. 2B, Table). Similar reductions were found in the Brn-3b cell density in the treated eyes (Fig. 2B, Table). Second, we estimated RGC loss by comparing the number of axons of untreated and treated eyes (Fig. 2C). The axonal loss in hypertensive eyes gradually increased from 13% at 1 week, to 21% at 2 weeks, to 27% at 4 weeks, to 33% at 8 weeks, and to 37% at 12 weeks after laser treatment (Fig. 2C, Table). The progressive loss of axons also strongly argues that the RGC loss was due to the sustained IOP elevation, rather than damage caused directly by laser. Third, because a major goal of ours was to investigate how dendritic structural changes precede RGC loss in Thy-1-YFP transgenic mice, we counted the total number of Thy-1-YFP-labeled RGC somas at 2 months after laser treatment. We found a 27% decrease in Thy-1-YFP-positive cells in the laser-treated retinas (treated: 38 ± 4 cells/retina; untreated: 52 ± 3 cells/retina, $P = 0.009$ in Student's *t*-test). Finally, we examined RGCs immunostained by the SMI-32 antibody, which selectively marks three to four subtypes of RGCs, including large alpha-type RGCs.^{10,42,43} At 2 months after laser treatment, a 21% decrease in SMI-32 cell density was found in laser-treated eyes ($11.5 \pm 0.3/10^4 \mu\text{m}^2$) compared to the untreated eyes ($14.6 \pm 0.9/10^4 \mu\text{m}^2$, $P = 0.003$ in Student's *t*-test). Although all methods revealed a progressive loss of RGCs, the degree of loss varied with method, perhaps suggesting that particular subtypes of RGC may be more susceptible to IOP elevation than others.

We next examined whether retinal neurons other than RGCs were affected by IOP elevation (Fig. 2D). Retinal sections were prepared at 2 months after laser treatment and immunostained with antibodies of PKC α , a marker for rod bipolar cells, and calretinin, a marker for some subtypes of amacrine cells. Our data showed that the overall morphology of bipolar cells and amacrine cells was unaffected by IOP elevation (Fig. 2D). We also examined the inner retina with antibodies for VGLUT1, a synaptic protein found in bipolar cells; calbindin, a marker for horizontal and some subtypes of amacrine cells; and Gad65, a marker for GABAergic amacrine cells, and found no obvious changes in retinas from ocular

TABLE. Summary of RGC Loss after Laser-Induced Ocular Hypertension

Percentage of Decrease (Mean $\pm$ SEM)	Time after Laser					
	1 week	2 week	1 month	2 months	3 months	6 months
Axon	12.9% $\pm$ 0.4% (n = 6)	20.5% $\pm$ 1.6% (n = 6)	26.9% $\pm$ 0.9% (n = 6)	32.5% $\pm$ 0.9% (n = 6)	36.8% $\pm$ 1.0% (n = 5)	—
Bm-3a	—	—	9.1% $\pm$ 0.3% (n = 6)	16.1% $\pm$ 0.4% (n = 5)	—	19.7% $\pm$ 1.3% (n = 7)
Bm-3b	—	—	10.5% $\pm$ 0.5% (n = 6)	16.8% $\pm$ 0.4% (n = 7)	—	19.3% $\pm$ 0.6% (n = 8)
Thy-1	—	—	—	27.4% $\pm$ 3.9% (n = 10)	—	—
SMI-32	—	—	—	21.1% $\pm$ 2.6% (n = 4)	—	—

hypertensive eyes (data not shown). Our data were consistent with previous findings that cell loss and changes in cell morphology due to hypertension are limited to RGCs.²¹ One can infer from this, therefore, that changes in visual performance with ocular hypertension result from RGC degeneration and loss.

Decrease of Visual Acuity and Contrast Sensitivity with IOP Elevation

The optomotor test measures aspects of spatial vision via reflexive head-tracking movements and the two eyes can be tested separately, which greatly facilitated our experiments because we laser-treated one eye of the target mouse and left the other intact as the control. We tested only those animals with no obvious ocular abnormality or inflammation in the anterior chamber at 2 months after laser treatment (Figs. 3A, 1B, 2D). Before the laser treatment, there was no difference between the eyes and both exhibited normal acuity (left: 0.417 ± 0.006 cpd; right: 0.413 ± 0.005 cpd; n = 28 mice, P = 0.58 in Student's t -test, Fig. 3B). At 2 months after laser treatment, the acuity of the right eyes (elevated IOP) decreased significantly compared with the left control eyes (left: 0.393 ± 0.016 cpd; right: 0.238 ± 0.029 cpd; n = 15 mice, P < 0.001, Fig. 3B). We found a further reduction in acuity of the eyes with elevated IOP at 5 to 6 months after laser treatment (left: 0.397 ± 0.011 cpd; right: 0.177 ± 0.029 cpd; n = 12 mice, P < 0.001, Fig. 3B). We also measured contrast sensitivity at 2 months after laser treatment and found that the right eyes with elevated IOP exhibited lower contrast sensitivity at 0.161 cpd (left: 11.26 ± 1.34 ; right: 5.87 ± 1.04 ; n = 8 mice, P = 0.003 in Student's t -test, Fig. 3C).

Based on the data presented in the Table, it would seem that 20% to 30% of the RGCs are lost in our mouse model of ocular hypertension by 2 months. Because visual field defects in glaucoma patients are correlated with the decrease of RGC density,⁴⁴ the change in acuity observed here is somewhat more than would be expected by the drop in RGC number. Hence, there is good reason to ask whether degenerating RGCs might also be contributing to the behavioral changes in visual performance. Because the dendritic structure determines an RGC's function in collecting and integrating information about the visual world through its synaptic drive, next we examined the structural and functional degeneration of RGCs that accompany visual impairments in mice with elevated IOP.

Functional Degeneration of RGCs after IOP Elevation

We characterized the visual responsiveness of RGCs in treated and untreated retinas using a 256-channel MEA system (Fig. 4). We laser-treated the right eyes of 1.5-month-old mice and then recorded the visual responses of RGCs at 1.5 m later (postnatal days 88 and 100). We recorded the RGC responses to the checkerboard stimulation (Fig. 4A) from hypertensive eyes (n = 1492 cells, 12 mice) and age-matched controls (n = 2755 cells, 18 mice). The STA was calculated for all cells by averaging all stimulus frames in a 1-second time window preceding a spike, thereby generating a video sequence of the mean effective stimulus for triggering a response in each RGC (see Materials and Methods). We modeled the cell's receptive field as a generalized two-dimensional Gaussian, where the pixel intensities of the STA were normalized using the mean and SD of the STA (Figs. 4B, 4C). We defined visual responsiveness to be when a cell's STA contrast contains an element that exceeds 4 SDs (Fig. 4C). Our data indicated that fewer cells were visually responsive in treated retinas (mean: $67.8\% \pm 7.1\%$, n = 12

FIGURE 4. Functional degeneration of RGCs after laser treatment. (A) Schematic checkerboard stimulation. Each checker projects onto a $100 \times 100\text{-}\mu\text{m}$ area of the retina and its luminance value is randomly chosen from a Gaussian distribution centered around the mean luminance level. The stimulus was updated at 30 frames per second. (B) The spatiotemporal receptive field of each cell was estimated through STA analysis. The *legend bar* indicates the contrast of each pixel in the STA in units of SD from the mean luminance. (C) The representation of the receptive field as a 1-dimensional Gaussian along the x (blue) and y (red) axes in (B). Cells in which the peak STA contrast exceeds 4 SDs (red dashed line) are labeled as visually responsive. (D) Percentages of visually responsive cells from untreated and treated eyes. $n = 7$, and $P = 0.05$ in Student's *t*-test. (E) RGCs from hypertensive eyes had smaller receptive field (RF) center sizes compared to controls ($P = 0.0037$ in Student's *t*-test, and $P = 0.0038$ in K-S Test).

retinas) compared with untreated controls ($83.1\% \pm 4.1\%$, $n = 18$ retinas, $P = 0.05$ in Student's *t*-test, Fig. 4D).

We further measured the RF center areas from visually responsive cells in both treated and untreated eyes. In control retinas, the mean RF center size of RGCs was $8.93 \pm 0.13 \times 10^3 \mu\text{m}^2$; $n = 2225$ cells, 18 retinas). By contrast, the mean RF center size of RGCs in hypertensive eyes was $8.29 \pm 0.16 \times 10^3 \mu\text{m}^2$, $n = 1056$ cells, 12 retinas), significantly smaller than controls ($P = 0.0037$ in Student's *t*-test, $P = 0.0038$ in K-S test, Fig. 4E). Taken together, our data suggest that ocular hypertensive mice had fewer visually responsive RGCs and that their RF center sizes also shrank compared with controls. We next examined the corresponding structural changes of RGCs in hypertensive eyes.

Examining RGC Dendritic Field and Soma Areas Using Thy-1-YFP Transgenic Mice

To investigate the structural degeneration of RGCs in mice with ocular hypertension, we used the Thy-1-YFP transgenic mouse model, which has a small number of most, if not all, subtypes of RGCs labeled.^{10,11,37,65} Confocal images were taken to examine the dendritic structures of a total of 329 RGCs in laser-treated eyes and compared with confocal images for 338 cells from untreated eyes ($n = 11$ mice, Fig. 5). Z-stack images of YFP-expressing RGCs were projected onto a two-dimensional image on which the dendritic field and soma areas were measured using National Institutes of Health ImageJ software (Fig. 5A). We found that the mean dendritic field area of all RGCs in the treated eyes decreased approximately 11% (mean of untreated: $46.5 \pm 1.6 \times 10^3 \mu\text{m}^2$; treated: $41.7 \pm 1.4 \times 10^3 \mu\text{m}^2$), although this difference did not reach statistical significance ($P = 0.34$ in K-S test, but $P = 0.02$ in Student's *t*-test, Fig. 5C), similar to the finding by Kalesnykas and his colleagues.⁶⁵ The mean soma area of all RGCs in the treated eyes also decreased approximately 7% (untreated: $225 \pm 4 \mu\text{m}^2$; treated: $210 \pm 5 \mu\text{m}^2$, $P = 0.10$, Fig. 5D). Because

previous studies suggested that RGC loss in glaucoma was topological and/or subtype specific,^{18,19,21} we next examined whether RGCs from particular areas of the retina or some subtypes of RGCs were especially vulnerable to the insult of ocular hypertension. Perhaps statistically significant differences between subsets of RGCs might be obscured when total populations were compared.

The RGC Dendritic Atrophy Starts from the Vertical Axis and Precedes Shrinkage of the Soma

We divided retinas into four quadrants, superior, inferior, nasal, and temporal, to examine the changes of RGC dendritic field and soma area from different regions at 2 months after laser treatment (Fig. 6). Comparing retinas from ocular hypertensive and control eyes, we found a significant decrease in RGC dendritic field sizes along the vertical axis (Fig. 6). In the superior quadrant, the dendritic fields subtended by the ensemble of RGCs decreased significantly (mean of untreated: $61.3 \pm 3.6 \times 10^3 \mu\text{m}^2$, 73 cells; treated: $47.0 \pm 2.8 \times 10^3 \mu\text{m}^2$, 68 cells; $P = 0.03$ in K-S test, same below). A corresponding decrease was observed in the inferior quadrant (mean of untreated: $48.0 \pm 3.5 \times 10^3 \mu\text{m}^2$, 66 cells; treated: $36.9 \pm 2.8 \times 10^3 \mu\text{m}^2$, 82 cells; $P = 0.005$). By contrast, the mean dendritic field area of RGCs in the nasal and temporal quadrants were alike statistically (nasal: $55.3 \pm 3.7 \times 10^3 \mu\text{m}^2$, 64 cells in untreated versus $61.3 \pm 3.9 \times 10^3 \mu\text{m}^2$, 54 cells in treated, $P = 0.17$; temporal: $30.3 \pm 1.3 \times 10^3 \mu\text{m}^2$, 116 cells in untreated versus $32.0 \pm 1.6 \times 10^3 \mu\text{m}^2$, 98 cells in treated, $P = 0.09$; Fig. 6).

We also observed a significant decrease in soma area in the superior quadrant but not in the inferior quadrant. In the superior quadrant, the RGC soma area shrank significantly (untreated: $227.9 \pm 10.3 \mu\text{m}^2$, 73 cells; treated: $192.5 \pm 9.3 \mu\text{m}^2$, 68 cells; $P = 0.009$). However, the change in the inferior quadrant was not significant statistically (untreated: $205.6 \pm 10.3 \mu\text{m}^2$, 66 cells; treated: $191.6 \pm 8.6 \mu\text{m}^2$, 82 cells; $P = 0.42$

FIGURE 5. At 2 months after laser treatment, the average dendritic field and soma areas of RGCs may become smaller. (A) A projected image of confocal Z-stacks of a Thy-1-YFP RGC shows the dendritic coverage area circled by a green circumferential line and the soma area by the orange line. Scale bar: 20 μm. (B) At 2 months after laser treatment, the dendritic field sizes of all RGCs from treated (red) and untreated (black) eyes were plotted against their soma sizes. (C) The relative cumulative distributions of the dendritic field sizes from treated (black) and untreated (gray) eyes. Separation of points: 10,000 μm². (D) The relative cumulative distributions of the soma size from treated and untreated eyes. Separation of points: 20 μm². *P* values were calculated by the K-S test (same in Figs. 6, 7).

in K-S test, Fig. 6). Our data suggest therefore that RGC degeneration begins along the vertical axis in our mouse model, first seen with changes in the dendritic tree and probably preceding shrinkage of the soma. The first occurrence of RGC dysfunction may be in the superior quadrant. Differences in the cumulative distributions shown in Figure 6 might indicate that, where changes are seen, there are many fewer RGCs with large dendritic trees. Is this because RGC subtypes with large dendritic trees are more susceptible to elevated IOP or is it that there is a shrinkage of dendritic trees, irrespective of cell type?

ON RGCs Are More Susceptible to IOP Elevation than ON-OFF RGCs

To examine whether some subtypes of RGCs are particularly vulnerable to degeneration with elevated IOP, we classified RGCs into three subtypes based on their dendritic lamination pattern in the inner plexiform layer (IPL; Figs. 7A, 7D). ON-OFF RGCs, responding to both light onset and offset, have bilaminated dendrites in the IPL; ON RGCs and OFF RGCs, which increase their firing rates in response to light ON and OFF in their receptive field centers have their dendrites monolaminated in the ON and OFF sublamina, respectively.^{10,37} We report results here only for ON and ON-OFF subtypes because we lacked a large enough sample of OFF cells. As we had found significant decreases in dendritic field area in the superior and inferior quadrants, we focused on cells from these two regions. At 2 months after laser treatment, ON RGCs exhibited a significant decrease in their dendritic field areas in the superior quadrant (mean of untreated: $78.7 \pm 5.4 \times 10^3 \mu\text{m}^2$, 35 cells; treated: $51.8 \pm 4.1 \times 10^3 \mu\text{m}^2$, 36 cells; *P* = 0.001 in K-S test) and in the inferior quadrant (untreated: 60.0

$\pm 4.9 \times 10^3 \mu\text{m}^2$, 36 cells; treated: $42.4 \pm 4.2 \times 10^3 \mu\text{m}^2$, 43 cells; *P* = 0.002, Fig. 7B). The cumulative distributions show that there is a dramatic loss of large dendritic trees. By contrast, no significant changes were found for ON-OFF RGCs in the superior quadrant (untreated: $43.3 \pm 4.0 \times 10^3 \mu\text{m}^2$, 24 cells; treated: $41.7 \pm 4.1 \times 10^3 \mu\text{m}^2$, 26 cells; *P* = 0.74) or in the inferior quadrant (untreated: $32.6 \pm 4.5 \times 10^3 \mu\text{m}^2$, 21 cells; treated: $30.3 \pm 4.1 \times 10^3 \mu\text{m}^2$, 30 cells; *P* = 0.83, Fig. 7E).

We also examined the soma shrinkage in the superior quadrant (Figs. 7C, 7F). The mean soma area of ON RGCs decreased significantly (untreated: $265.5 \pm 13.3 \mu\text{m}^2$, 35 cells; treated: $207.5 \pm 13.9 \mu\text{m}^2$, 36 cells; *P* = 0.017, Fig. 7C), but no significant change was observed for ON-OFF RGCs (untreated: $195.3 \pm 17.6 \mu\text{m}^2$, 24 cells; treated: $172.6 \pm 13.0 \mu\text{m}^2$, 26 cells; *P* = 0.36 in K-S test, Fig. 7F). Taken together, the data presented in Figures 5 to 7 show convincingly that both the degeneration of RGC dendritic structure and shrinkage in soma size depends on cell type and location.

SMI-32 Positive on RGCs in the Superior Quadrant Are Particularly Susceptible to IOP Elevation

We specifically investigated one type of RGC doubled-labeled by SMI-32 and Thy-1-YFP (Fig. 8A). The mean dendritic field size of all SMI-32-positive RGCs in treated eyes ($55.2 \pm 2.7 \times 10^3 \mu\text{m}^2$, 99 cells) was similar to that of the untreated eyes ($57.9 \pm 3.1 \times 10^3 \mu\text{m}^2$, 106 cells; *P* = 0.5 in K-S test); so was the mean soma area (untreated: $269.8 \pm 5.4 \mu\text{m}^2$; treated: $255.0 \pm 5.3 \mu\text{m}^2$, *P* = 0.08 in K-S test, Fig. 8B). Because ON RGCs of the superior quadrant exhibited significant dendritic shrinkage in hypertensive eyes, we further examined SMI-32-labeled ON cells of this area. This is a population of RGCs with comparatively large dendritic trees and large somas and we

FIGURE 6. The degeneration of RGC dendrites starts from the vertical axis and the shrinkage of RGC somas starts from the superior quadrant. After immunostaining, retinas were flat-mounted like clover leaves with four quadrants: superior, inferior, temporal, and nasal (labeled as the *dark area in the circle*). Thy-1-YFP-positive cells from different regions were measured and compared between treated (gray) and untreated (black) eyes. Scale bar: 500 μm .

found that their mean dendritic field area decreased significantly in the superior quadrant of hypertensive eyes ($66.3 \pm 6.0 \times 10^3 \mu\text{m}^2$, 18 cells) in comparison with untreated eyes ($93.1 \pm 8.2 \times 10^3 \mu\text{m}^2$, 16 cells; $P = 0.01$ in Student's *t*-test, Fig. 8C). At the same time, we found that the mean soma area of SMI-32-positive ON RGCs in the superior quadrant was unaffected in treated retinas (untreated: $280.2 \pm 15.4 \mu\text{m}^2$; treated: $255.5 \pm 14.4 \mu\text{m}^2$, $P = 0.4$ in Student's *t*-test, Fig. 8C), again indicating that RGC dendritic degeneration precedes shrinkage of the soma.

Because SMI-32-positive ON RGCs in the superior quadrant exhibited significant shrinkage in eyes with elevated IOP, we further traced their dendrites (Fig. 8D). We find a 13% decrease of the total dendritic length (treated: $4834 \pm 333 \mu\text{m}$, 16 cells) in comparison with untreated eyes ($5568 \pm 601 \mu\text{m}$, 10 cells, $n = 6$ retinas; $P = 0.05$ in Student's *t*-test, Fig. 8E). The branch number also decreased (untreated: 82 ± 7 versus treated: 74 ± 4), but this change was not significant statistically ($P = 0.34$ in Student's *t*-test). The results that we have obtained with the SMI-32-positive ON cells points strongly to the conclusion that RGCs with large dendritic trees may undergo degeneration early following elevated IOP.

DISCUSSION

Models of Experimental Glaucoma: From IOP Elevation to RGC Loss

Animal models of glaucoma have shifted from primates to rodents in recent decades because mouse models offer more potential to examine the function of critical genes and signaling pathways *in vivo*.^{45,46} Acute or chronic ocular hypertension in mice has been achieved by laser photocoagulating the outflow of aqueous humor,^{4,5,31} as we have done here, by obstruction of the meshwork with polystyrene beads,^{6,65} or by episcleral venous occlusion.^{47–49} For example, one injection of microbeads into mouse eyes can elicit an approximately 30% elevation in IOP for a couple of weeks,⁶ whereas another study suggested a longer effect of IOP elevation.^{50,65} The occlusion of limbal and episcleral veins in albino CD-1 mice induced acute IOP elevation for approximately 1 week.⁴⁹ By contrast, we report above that in our laser-induced ocular hypertension model, IOP almost doubled for approximately 4 months (Fig. 1), permitting us to measure loss of RGCs and declines in visual acuity over time (Figs. 2, 3). The sustained IOP elevation without significant inflammation or

FIGURE 7. ON RGCs are more susceptible to IOP elevation than ON-OFF RGCs. (A, D) Representative images of an ON-RGC (A) and an ON-OFF-RGC (D). The orthogonal views at the bottom show that ON cell dendrites were laminated in the ON sublamina and the ON-OFF dendritic tree in both ON and OFF sublaminae. Scale bar: 20 μm . (B, E) The relative cumulative distributions of the dendritic field sizes of ON cells (B) and ON-OFF cells (E) from superior and inferior quadrants. (C, F) The relative cumulative distributions of the soma sizes of ON cells (C) and ON-OFF cells (F) from superior quadrant. Untreated: black; and Treated: gray.

damage to other parts of the eyes makes our model particularly useful for studies of the long-term effects of ocular hypertension on RGC structure and function.

The RGC loss induced by IOP elevation varied among different animal models, which is probably due to the variability of the length and degree of IOP elevation. For

example, a 20% loss of axons has been observed in mouse eyes with an approximately 30% elevation in IOP induced by injection of microbeads.⁶ In DBA/2J mice, which develop glaucomalike symptoms with age,^{45,46} a 13.4% decrease in axon density was found in 13-month-old mice compared with 3-month-old mice, whereas a 75% reduction in retrogradely labeled

FIGURE 8. SMI-32-positive ON cells from the superior quadrant exhibited a smaller mean dendritic field size. (A) Immunostaining of SMI-32 antibody and double-staining of SMI-32 and YFP/GFP antibodies on retinal sections. Scale bar: 100 μm (left); scale grid: 20 μm (right). (B) The relative cumulative distributions of the dendritic field and soma sizes of all SMI-32 positive cells. P values were calculated by the K-S test. (C) The relative cumulative distributions of the dendritic field and soma sizes of SMI-32-labeled ON cells in the superior quadrant. (D) Dendritic tracing of an SMI-32- and Thy-1-YFP-positive RGC. Scale grid: 20 μm . (E) The total dendritic length of SMI-32- and Thy-1-YFP-positive RGCs from the superior quadrant of treated and untreated retinas. P = 0.05 in Student's *t*-test.

RGCs has been reported in these aged DBA mice.⁵¹ In our laser-induced model of chronic ocular hypertension, we have seen a progressive loss of axons with time, from 13% at 1 week, to 37% at 12 weeks after laser treatment (Fig. 2C). Different labeling methods might also have contributed to the differences observed in RGC loss between different animal models. For example, by immunostaining data with different subtype markers for RGCs, one may expect to review subtype differences in susceptibility to IOP elevation (see Figs. 2, 7, 8, and discussion below). By contrast, the retrograde labeling of RGCs from the superior colliculus could potentially survey the loss of most RGCs, this latter approach might overestimate RGC loss due to the disruption of axonal transport in glaucoma.^{51,52}

The molecular pathways mediating pressure-induced RGC degeneration and death are poorly understood.⁵³ High pressure may place stress on the optic nerve head, leading to axonal degeneration and then RGC death, although evidence supporting this hypothesis is inconclusive.^{2,54} Although apoptosis is known to contribute to RGC death following IOP elevation,^{55,56} it is unknown where RGCs are most susceptible to a pressure insult. Intrinsic axonal degeneration pathways are different from those of classical somatic apoptotic pathways.^{57,58} For example, the pro-apoptotic molecule BCL2-associated X protein is required for RGC death, but not for RGC axon degeneration in inherited DBA/2J glaucoma.⁵⁹ By contrast, the Wallerian degeneration slow (*Wld^s*) allele significantly delays axonal degeneration, but not somatic shrinkage.^{27,60} Other findings suggest that dendritic pathology occurs early in the disease, but little is known of the underlying mechanisms.^{8,14,30} Combined with the power of mouse genetics, the characterization of RGC degeneration presented in this article provides a superb readout with which to investigate the molecular signaling that leads to RGC dendritic degeneration and vision loss in high-tension glaucoma.

Susceptibility of RGCs to Glaucomatous Damage

It remains controversial whether different RGC subtypes respond to the injury in glaucoma differentially.^{21,23,24} Studies have been limited to low-resolution general observations of RGC morphological changes, which is partially due to the lack of subtype-specific markers and technical difficulties in labeling RGCs and their dendrites in vivo. Using transgenic Thy-1-YFP mice, which have most, if not all, subtypes of RGCs and their dendrites labeled, we could sample hundreds of cells from glaucomatous eyes. As a first, admittedly incomplete classification scheme, we subdivided RGCs into ON, OFF, and ON-OFF subgroups based on their dendritic lamination pattern in the IPL (Figs. 5, 7). We find that ON RGCs have approximately a 23% reduction (Fig. 7) and a specific type of ON RGCs labeled by SMI-32 approximately a 34% reduction in their dendritic tree size in the affected areas of hypertensive eyes (Fig. 8). In monkey glaucomatous eyes, approximately a 40% reduction in the mean dendritic field size of parasol cells was observed.²² Because glaucoma results in progressive damage of RGCs, these numerical differences may simply reflect where in the time line of degeneration each snapshot was taken, in addition to the differences in RGC subtype vulnerability.

The complexity of dendritic trees is also significantly reduced in glaucoma.^{24,30} Parasol cells from glaucomatous eyes had smaller and less complex dendritic arbors, resulting in a significant reduction in total dendrite length.²⁴ We observed some RGCs with abnormal morphology, such as contorted dendrites that seemed to loop and fold back, and that some RGCs had just their primary dendrites, sometimes just the

proximal segments (see Supplementary Material and Supplementary Fig. S2, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10791/-DCSupplemental>). Similar phenotypes have been observed in glaucoma patients post mortem.⁶¹

In addition, RGC damage is nonuniformly distributed across the retina,² which adds another layer of complication. For example, glaucoma patients show deficits in motion sensitivity that are most pronounced in the superior field at 15- and 21-degree eccentricity.⁶² In DBA/2J mice, RGC degeneration is equally severe at all eccentricities, but regions of cell death radiate from the optic nerve head in fan-shaped sectors.²¹ Our data show that SMI-32-labeled large ON RGCs in the superior retina are more susceptible to elevated IOP (Fig. 8), supporting the notion that RGC degeneration is both subtype- and location-dependent. Because glaucoma is a disease in which cell loss is progressive and whose impact varies with retinal location, it is little wonder that there might be differences in reported damage based on the severity of the insult and the retinal location probed. Without a careful systematic approach of the type we have followed here, where location and cell types are segregated, changes might easily be missed.

How Does Functional Degeneration Correlate with the Structural Degeneration of RGCs?

It is not well understood how abnormalities in RGC structure and function might translate into visual impairments. In the primate model of experimental glaucoma, RGC losses are correlated with the visual field defects.^{63,64} With our mouse model, we also observed a significant RGC loss (Fig. 2) accompanied with a decrease in visual acuity and contrast sensitivity (Fig. 3). We further examined how the dendritic structure and visual response properties of RGCs changed in parallel with behavioral changes in visual performance. We find that large ON cells labeled by SMI-32 from the superior quadrant had smaller dendritic tree area and shorter dendrites in ocular hypertension than control animals. Our results correspond with previous findings from a cat model of chronic ocular hypertension in which alpha RGCs exhibited dendritic shrinkage and cell loss.²³ Presumptive parasol cells in glaucomatous eyes are reported to exhibit smaller and less complex dendritic trees, and generate abnormal responses to grating stimuli.²⁴ With the dendritic atrophy of ON cells in hypertensive eyes, we might expect that their receptive field center sizes would decrease too. We are currently developing analytical methods to characterize different RGC types physiologically to correlate changes in dendritic structure with changes in the visual receptive field properties of individual RGCs more directly.

Acknowledgments

We thank Sarah Lindstrom for her technical support for the MEA recordings, Thomas Bozza for sharing his confocal microscope, Genn Suyeoka for genotyping, and Angelo P. Tanna for his helpful comments on this manuscript. Part of the axon-counting experiment in Figure 2C was done by Liang Feng while in the laboratory of Rosario Hernandez-Neufeld (deceased).

References

- Gupta N, Yucel YH. Glaucoma as a neurodegenerative disease. *Curr Opin Ophthalmol*. 2007;18:110–114.
- Quigley HA. Neuronal death in glaucoma. *Prog Retin Eye Res*. 1999;18:39–57.
- McKinnon SJ, Schlamp CL, Nickells RW. Mouse models of retinal ganglion cell death and glaucoma. *Exp Eye Res*. 2009; 88:816–824.

4. Aihara M, Lindsey JD, Weinreb RN. Experimental mouse ocular hypertension: establishment of the model. *Invest Ophthalmol Vis Sci.* 2003;44:4314–4320.
5. Grozdanic SD, Betts DM, Sakaguchi DS, Allbaugh RA, Kwon YH, Kardon RH. Laser-induced mouse model of chronic ocular hypertension. *Invest Ophthalmol Vis Sci.* 2003;44:4337–4346.
6. Sappington RM, Carlson BJ, Crish SD, Calkins DJ. The microbead occlusion model: a paradigm for induced ocular hypertension in rats and mice. *Invest Ophthalmol Vis Sci.* 2010;51:207–216.
7. Schlamp CL, Li Y, Dietz JA, Janssen KT, Nickells RW. Progressive ganglion cell loss and optic nerve degeneration in DBA/2J mice is variable and asymmetric. *BMC Neurosci.* 2006;7:66.
8. Nickells RW. From ocular hypertension to ganglion cell death: a theoretical sequence of events leading to glaucoma. *Can J Ophthalmol.* 2007;42:278–287.
9. Sernagor E, Eglén SJ, Wong RO. Development of retinal ganglion cell structure and function. *Prog Retin Eye Res.* 2001;20:139–174.
10. Liu X, Grishanin RN, Tolwani RJ, et al. Brain-derived neurotrophic factor and TrkB modulate visual experience-dependent refinement of neuronal pathways in retina. *J Neurosci.* 2007;27:7256–7267.
11. Sun W, Li N, He S. Large-scale morphological survey of mouse retinal ganglion cells. *J Comp Neurol.* 2002;451:115–126.
12. Troy JB, Shou T. The receptive fields of cat retinal ganglion cells in physiological and pathological states: where we are after half a century of research. *Prog Retin Eye Res.* 2002;21:263–302.
13. Volgyi B, Chheda S, Bloomfield SA. Tracer coupling patterns of the ganglion cell subtypes in the mouse retina. *J Comp Neurol.* 2009;512:664–687.
14. Liu M, Duggan J, Salt TE, Cordeiro MF. Dendritic changes in visual pathways in glaucoma and other neurodegenerative conditions. *Exp Eye Res.* 2011;92:244–250.
15. Quigley HA, Sanchez RM, Dunkelberger GR, L'Hernault NL, Baginski TA. Chronic glaucoma selectively damages large optic nerve fibers. *Invest Ophthalmol Vis Sci.* 1987;28:913–920.
16. Quigley HA, Dunkelberger GR, Green WR. Chronic human glaucoma causing selectively greater loss of large optic nerve fibers. *Ophthalmology.* 1988;95:357–363.
17. Morgan JE. Retinal ganglion cell shrinkage in glaucoma. *J Glaucoma.* 2002;11:365–370.
18. Filippopoulos T, Danias J, Chen B, Podos SM, Mittag TW. Topographic and morphologic analyses of retinal ganglion cell loss in old DBA/2N^{nia} mice. *Invest Ophthalmol Vis Sci.* 2006;47:1968–1974.
19. Reichstein D, Ren L, Filippopoulos T, Mittag T, Danias J. Apoptotic retinal ganglion cell death in the DBA/2 mouse model of glaucoma. *Exp Eye Res.* 2007;84:13–21.
20. Danias J, Lee KC, Zamora MF, et al. Quantitative analysis of retinal ganglion cell (RGC) loss in aging DBA/2N^{nia} glaucomatous mice: comparison with RGC loss in aging C57/BL6 mice. *Invest Ophthalmol Vis Sci.* 2003;44:5151–5162.
21. Jakobs TC, Libby RT, Ben Y, John SW, Masland RH. Retinal ganglion cell degeneration is topological but not cell type specific in DBA/2J mice. *J Cell Biol.* 2005;171:313–325.
22. Weber AJ, Kaufman PL, Hubbard WC. Morphology of single ganglion cells in the glaucomatous primate retina. *Invest Ophthalmol Vis Sci.* 1998;39:2304–2320.
23. Shou T, Liu J, Wang W, Zhou Y, Zhao K. Differential dendritic shrinkage of alpha and beta retinal ganglion cells in cats with chronic glaucoma. *Invest Ophthalmol Vis Sci.* 2003;44:3005–3010.
24. Weber AJ, Harman CD. Structure-function relations of parasol cells in the normal and glaucomatous primate retina. *Invest Ophthalmol Vis Sci.* 2005;46:3197–3207.
25. Fiorentini A, Maffei L, Pirchio M, Spinelli D, Porciatti V. The ERG in response to alternating gratings in patients with diseases of the peripheral visual pathway. *Invest Ophthalmol Vis Sci.* 1981;21:490–493.
26. Hood DC, Xu L, Thienprasiddhi P, et al. The pattern electroretinogram in glaucoma patients with confirmed visual field deficits. *Invest Ophthalmol Vis Sci.* 2005;46:2411–2418.
27. Howell GR, Libby RT, Jakobs TC, et al. Axons of retinal ganglion cells are insulted in the optic nerve early in DBA/2J glaucoma. *J Cell Biol.* 2007;179:1523–1537.
28. Weber AJ, Harman CD, Viswanathan S. Effects of optic nerve injury, glaucoma, and neuroprotection on the survival, structure, and function of ganglion cells in the mammalian retina. *J Physiol.* 2008;586:4393–4400.
29. Luo X, Patel NB, Harwerth RS, Frishman LJ. Loss of the low-frequency component of the global-flash multifocal electroretinogram in primate eyes with experimental glaucoma. *Invest Ophthalmol Vis Sci.* 2011;52:3792–3804.
30. Morgan JE, Datta AV, Erichsen JT, Albon J, Boulton ME. Retinal ganglion cell remodelling in experimental glaucoma. *Adv Exp Med Biol.* 2006;572:397–402.
31. Levkovitch-Verbin H, Quigley HA, Martin KR, Valenta D, Baumrind LA, Pease ME. Translimbal laser photocoagulation to the trabecular meshwork as a model of glaucoma in rats. *Invest Ophthalmol Vis Sci.* 2002;43:402–410.
32. Rangarajan KV, Lawhn-Heath C, Feng L, Kim TS, Cang J, Liu X. Detection of visual deficits in aging DBA/2J mice by two behavioral assays. *Curr Eye Res.* 2011;36:481–491.
33. John SW, Hagaman JR, MacTaggart TE, Peng L, Smithes O. Intraocular pressure in inbred mouse strains. *Invest Ophthalmol Vis Sci.* 1997;38:249–253.
34. Wang L, Rangarajan KV, Lawhn-Heath CA, et al. Direction-specific disruption of subcortical visual behavior and receptive fields in mice lacking the beta2 subunit of nicotinic acetylcholine receptor. *J Neurosci.* 2009;29:12909–12918.
35. Douglas RM, Alam NM, Silver BD, McGill TJ, Tschetter WW, Prusky GT. Independent visual threshold measurements in the two eyes of freely moving rats and mice using a virtual-reality optokinetic system. *Vis Neurosci.* 2005;22:677–684.
36. Prusky GT, Alam NM, Beekman S, Douglas RM. Rapid quantification of adult and developing mouse spatial vision using a virtual optomotor system. *Invest Ophthalmol Vis Sci.* 2004;45:4611–4616.
37. Liu X, Robinson ML, Schreiber AM, et al. Regulation of neonatal development of retinal ganglion cell dendrites by neurotrophin-3 overexpression. *J Comp Neurol.* 2009;514:449–458.
38. Cantrell DR, Cang J, Troy JB, Liu X. Non-centered spike-triggered covariance analysis reveals neurotrophin-3 as a developmental regulator of receptive field properties of ON-OFF retinal ganglion cells. *PLoS Comput Biol.* 2010;6:e1000967.
39. Pinto LH, Vitaterna MH, Shimomura K, et al. Generation, identification and functional characterization of the nob4 mutation of Grm6 in the mouse. *Vis Neurosci.* 2007;24:111–123.
40. Chichilnisky EJ. A simple white noise analysis of neuronal light responses. *Network.* 2001;12:199–213.
41. Devries SH, Baylor DA. Mosaic arrangement of ganglion cell receptive fields in rabbit retina. *J Neurophysiol.* 1997;78:2048–2060.
42. Nixon RA, Lewis SE, Dahl D, Marotta CA, Drager UC. Early posttranslational modifications of the three neurofilament subunits in mouse retinal ganglion cells: neuronal sites and

- time course in relation to subunit polymerization and axonal transport. *Brain Res Mol Brain Res*. 1989;5:93–108.
43. Coombs J, van der List D, Wang GY, Chalupa LM. Morphological properties of mouse retinal ganglion cells. *Neuroscience*. 2006;140:123–136.
 44. Harwerth RS, Wheat JL, Fredette MJ, Anderson DR. Linking structure and function in glaucoma. *Prog Retin Eye Res*. 2010;29:249–271.
 45. Libby RT, Gould DB, Anderson MG, John SW. Complex genetics of glaucoma susceptibility. *Annu Rev Genomics Hum Genet*. 2005;6:15–44.
 46. Howell GR, Libby RT, John SW. Mouse genetic models: an ideal system for understanding glaucomatous neurodegeneration and neuroprotection. *Prog Brain Res*. 2008;173:303–321.
 47. Shareef SR, Garcia-Valenzuela E, Salierno A, Walsh J, Sharma SC. Chronic ocular hypertension following episcleral venous occlusion in rats. *Exp Eye Res*. 1995;61:379–382.
 48. Chiu K, Chang R, So KF. Laser-induced chronic ocular hypertension model on SD rats. *J Vis Exp*. 2007;10:549.
 49. Fu CT, Sretavan D. Laser-induced ocular hypertension in albino CD-1 mice. *Invest Ophthalmol Vis Sci*. 2010;51:980–990.
 50. Ding C, Wang P, Tian N. Effect of general anesthetics on IOP in elevated IOP mouse model. *Exp Eye Res*. 2011;92:512–520.
 51. Buckingham BP, Inman DM, Lambert W, et al. Progressive ganglion cell degeneration precedes neuronal loss in a mouse model of glaucoma. *J Neurosci*. 2008;28:2735–2744.
 52. Quigley HA, McKinnon SJ, Zack DJ, et al. Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. *Invest Ophthalmol Vis Sci*. 2000;41:3460–3466.
 53. Nickells RW, Howell GR, Soto I, John SW. Under pressure: cellular and molecular responses during glaucoma, a common neurodegeneration with axonopathy. *Annual Rev Neurosci*. 2012;35:153–179.
 54. Quigley HA, Addicks EM, Green WR, Maumenee AE. Optic nerve damage in human glaucoma. II. The site of injury and susceptibility to damage. *Arch Ophthalmol*. 1981;99:635–649.
 55. Kerrigan LA, Zack DJ, Quigley HA, Smith SD, Pease ME. TUNEL-positive ganglion cells in human primary open-angle glaucoma. *Arch Ophthalmol*. 1997;115:1031–1035.
 56. Nickells RW. Apoptosis of retinal ganglion cells in glaucoma: an update of the molecular pathways involved in cell death. *Surv Ophthalmol*. 1999;43:S151–S161.
 57. Coleman MP, Perry VH. Axon pathology in neurological disease: a neglected therapeutic target. *Trends Neurosci*. 2002;25:532–537.
 58. Raff MC, Whitmore AV, Finn JT. Axonal self-destruction and neurodegeneration. *Science*. 2002;296:868–871.
 59. Libby RT, Li Y, Savinova OV, et al. Susceptibility to neurodegeneration in a glaucoma is modified by Bax gene dosage. *PLoS Genet*. 2005;1:17–26.
 60. Beirowski B, Babetto E, Coleman MP, Martin KR. The WldS gene delays axonal but not somatic degeneration in a rat glaucoma model. *Eur J Neurosci*. 2008;28:1166–1179.
 61. Pavlidis M, Stupp T, Naskar R, Cengiz C, Thanos S. Retinal ganglion cells resistant to advanced glaucoma: a postmortem study of human retinas with the carbocyanine dye DiI. *Invest Ophthalmol Vis Sci*. 2003;44:5196–5205.
 62. Joffe KM, Raymond JE, Chrichton A. Motion coherence perimetry in glaucoma and suspected glaucoma. *Vis Res*. 1997;37:955–964.
 63. Harwerth RS, Carter-Dawson L, Shen F, Smith EL III, Crawford ML. Ganglion cell losses underlying visual field defects from experimental glaucoma. *Invest Ophthalmol Vis Sci*. 1999;40:2242–2250.
 64. Harwerth RS, Smith EL III, Chandler M. Progressive visual field defects from experimental glaucoma: measurements with white and colored stimuli. *Optom Vis Sci*. 1999;76:558–570.
 65. Kalesnykas G, Oglesby EN, Zack DJ, et al. Retinal ganglion cell morphology after optic nerve crush and experimental glaucoma. *Invest Ophthalmol Vis Sci*. 2012;53:3847–3857.