

# Quantification of the Effect of Different Levels of IOP in the Astroglia of the Rat Retina Ipsilateral and Contralateral to Experimental Glaucoma

Ana I. Ramírez,<sup>1</sup> Juan J. Salazar,<sup>1</sup> Rosa de Hoz,<sup>1</sup> Blanca Rojas,<sup>1</sup> Beatriz I. Gallego,<sup>1</sup> Manuel Salinas-Navarro,<sup>2</sup> Luis Alarcón-Martínez,<sup>2</sup> Arturo Ortín-Martínez,<sup>2</sup> Marcelino Avilés-Trigueros,<sup>2</sup> Manuel Vidal-Sanz,<sup>2</sup> Alberto Triviño,<sup>1</sup> and Jose M. Ramírez<sup>1</sup>

**PURPOSE.** To analyze the effects of different levels of intraocular pressure (IOP) in the macroglia in ocular hypertension (OHT) and contralateral eyes at 3 weeks after laser photocoagulation and compare these with effects in age-matched control rats.

**METHODS.** Adult Sprague-Dawley rats were divided into an age-matched control (naïve) group and an OHT group. Retinas were processed as whole mounts and immunostained with GFAP for analysis of the retinal macroglia.

**RESULTS.** The area of the retina occupied by astrocytes (AROA) was quantified. GFAP immunostaining showed common features in ipsilateral and contralateral eyes. First, although the astrocyte network maintained a star-shaped morphology, these cells had fewer secondary processes and thinner cell bodies and primary processes than did naïve cells. Second, Müller cells appeared as punctate GFAP+ structures among astrocytes. Third, there was a significant reduction of the AROA in ipsilateral and contralateral eyes compared with naïve eyes. Ipsilateral eyes had significantly less AROA than did contralateral eyes. The decrease was greater for OHT eyes with higher IOP levels.

**CONCLUSIONS.** OHT induces changes in the macroglia of contralateral eyes; thus, these fellow eyes should not be used as control. In eyes with OHT, there is a close relationship between IOP values and decreased AROA. (*Invest Ophthalmol Vis Sci.* 2010;51:5690–5696) DOI:10.1167/iops.10-5248

It is known that several mechanisms participate in the death of retinal ganglion cells (RGCs) in glaucoma<sup>1–8</sup>; however, the physiopathological mechanisms involved in RGC death

remain poorly understood.<sup>9,10</sup> During recent years, several reports on glial behavior in ocular hypertension (OHT) and ischemia have suggested that in some diseases, such as primary open-angle glaucoma, glial cells may be involved in RGC dysfunction.<sup>11</sup>

Under normal conditions, Müller cells appear to participate in the maintenance of RGC survival by mechanisms only partially known.<sup>12,13</sup> Astrocytes are an abundant cell type in the optic nerve and the retina that are intercommunicated with the neurons and the surrounding connective tissue through their microenvironment, and together these components function as a unit.<sup>14</sup> Astrocytes participate in the detoxification and in the structural and metabolic support<sup>15</sup> of the nervous system and neuronal protectors during the aging process.<sup>16</sup>

Under pathologic conditions, such as glaucoma, there is a reduction of the RGCs and their axons in addition to a decrease in neural cells in the lateral geniculated nucleus and the visual cortex.<sup>17</sup> Such a scenario, which is associated with a glial response that varies depending on whether the cell is astroglia or Müller glia, can be reproduced in experimental glaucoma.

Astrocytes are known to have the capacity to regulate the immune response in the central nervous system,<sup>18,19</sup> the retina, and the optic nerve. In the eye, Müller cells also participate in the immune response.<sup>20</sup> In glaucoma, glial reactivity is associated with an upregulation of class II molecules of the major histocompatibility complex (MHC).<sup>21</sup> The immune response could be protective or destructive, depending on whether there is efficient control of the intrinsic immunoregulatory mechanism,<sup>22</sup> and could explain the glial reactivity observed in the contralateral eyes of animals with unilaterally induced experimental glaucoma.<sup>23</sup>

As discussed, glaucomatous eyes lose RGCs and have a glial response that is also apparent in the contralateral retina. However, it is not well established whether there is any relationship between different levels of OHT and the magnitude of the OHT-induced changes in the population of retinal astrocytes.

The aim of the present work, using a rat model of laser-induced OHT, was to analyze the effects of OHT on retinal astrocytes and Müller cell populations in the treated eye, the changes in retinal macroglia in the contralateral-fellow untreated eyes, and any relationship between different levels of OHT and the magnitude of the OHT-induced changes in the population of astrocytes in both lasered and contralateral untreated eyes.

From the <sup>1</sup>Instituto de Investigaciones Oftalmológicas Ramón Castroviejo, Universidad Complutense, Madrid, Spain; and the <sup>2</sup>Departamento de Oftalmología, Facultad de Medicina, Universidad de Murcia, Murcia, Spain.

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Corresponding author: José M. Ramírez, Instituto de Investigaciones Oftalmológicas Ramón Castroviejo. School of Medicine, Complutense University, 28040 Madrid, Spain; ramirezsm@med.ucm.es.

## MATERIALS AND METHODS

### Animals and Anesthetics

Female albino Sprague-Dawley (SD) adult (weight range, 180–200 g) rats obtained from the breeding colony of the University of Murcia (Murcia, Spain) were housed in temperature- and light-controlled rooms with a 12-hour light/12-hour dark cycle and had ad libitum access to food and water. Light intensity within the cages ranged from 9 to 24 lux. Animal manipulations followed institutional guidelines, European Union regulations for the use of animals in research, and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All surgical manipulations were carried out under general anesthesia induced with intraperitoneal (IP) injection of a mixture of ketamine (70 mg/kg, Ketalar; Parke-Davies, S.L., Barcelona, Spain) and xylazine (10 mg/kg, Rompun; Bayer, S.A., Barcelona, Spain). Animals were killed by IP injection of an overdose of pentobarbital (Dolethal; Vétoquinol, Especialidades Veterinarias, S.A., Alcobendas, Madrid, Spain).<sup>9</sup>

### Induction of Ocular Hypertension and IOP Measurements

The left eyes were treated in a single session with diode laser burns (Viridis Ophthalmic Photocoagulator 532-nm laser; Quantel Medical, Clermont-Ferrand, France), as recently described in detail.<sup>9</sup> In brief, the laser beam was directly delivered on anesthetized rats without any lenses and was aimed at the trabecular meshwork and the perilimbal and episcleral veins. The spot size, duration, and power used were 50 to 100  $\mu\text{m}$ , 0.5 seconds, and 0.4 W, respectively. Each rat received between 65 and 90 burns.

IOP was measured in both eyes with a tonometer (Tono-Pen XL; Reichert Ophthalmic Instruments Depew, NY)<sup>24,25</sup> while rats were under anesthesia (Colircusí anestésico doble; Alcón Cusí, S.A., Barcelona, Spain) before and 1 and 2 weeks after laser photocoagulation (LP) in those with OHT and before kill in age-matched controls.

At each time point, 8 to 12 consecutive readings were carried out for each eye and were averaged. To avoid fluctuations of the IOP because of the circadian rhythm<sup>25–27</sup> or because of elevation of the IOP itself,<sup>28</sup> we tested IOP consistently around the same time, preferentially in the morning and directly after deep anesthesia in all rats (with OHT and age-matched control). Moreover, because general anesthesia lowers IOP in the rat, we measured the IOP of the treated eye as well as the contralateral intact fellow eye in all the experiments.

### Experimental Groups

Two groups of animals were considered for the study: an age-matched control group (naive,  $n = 10$ ) and a group designed to determine the effects of OHT on retinal macroglia (OHT,  $n = 14$ ). The OHT group was processed 3 weeks after LP.

### Immunohistochemistry

The rats were deeply anesthetized and perfused transcardially through the ascending aorta first with saline and then with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4).

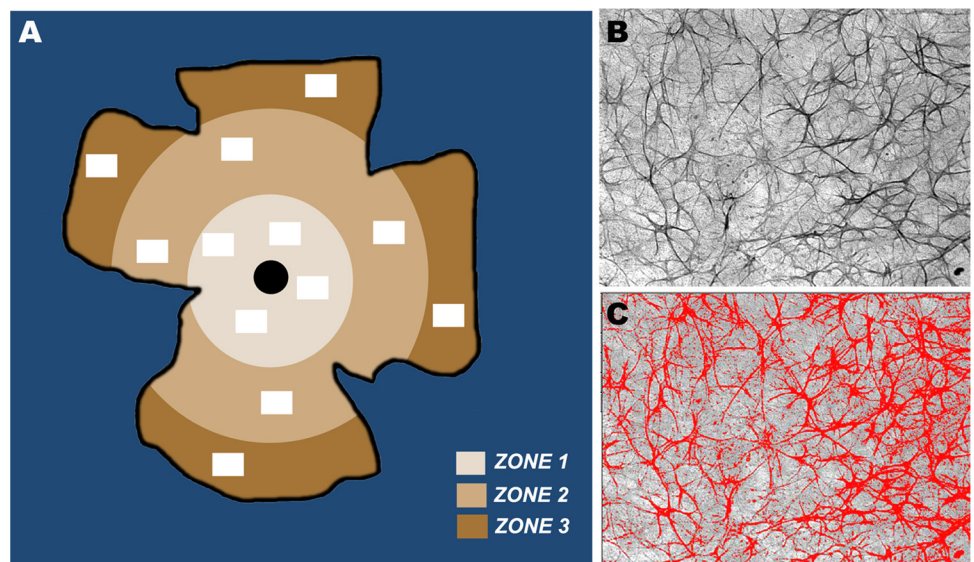
The retinas from both eyes were dissected and processed as whole mounts after the immunohistochemical protocol described elsewhere.<sup>29</sup> A monoclonal antibody against glial fibrillary acidic protein (GFAP clone GA-5; Sigma, St. Louis, MO) was used in a 1:250 dilution.

### Retinal Analysis: Area of the Retina Occupied by Astrocytes

Retinal astrocytes are interconnected, forming a network (Ramírez JM, et al. *IOVS* 2005;46:ARVO E-Abstract 1318).<sup>29</sup> This situation hampers the differentiation of individual cells for cell counting, leading us to consider the area of the retina occupied by astrocytes (AROA) to be a suitable zone to quantify rat retinal astroglia.

To quantify the AROA, we used a computer-assisted morphometric analysis system (Metamorph Imaging System, version 5; Universal Imaging Corp., Downingtown, PA) in association with an imaging microscope (Axioplan 2; Zeiss, Göttingen, Germany). For the study, each retinal whole mount was divided into three zones that extended concentrically from the optic nerve to the periphery as follows: central (zone 1), intermediate (zone 2), and peripheral (zone 3). Photomicrographs of four areas from each zone (12 areas per retina) were taken at random. The only selection criteria were good tissue quality, good staining, and clear visualization of astrocytes (Figs. 1A, 1B). Photographs were taken at 20 $\times$ , covering an area of 0.18890 mm<sup>2</sup> (Figs. 1B, 1C). Resultant images were processed with the Threshold Tool of the computer-assisted morphometric analysis system (Metamorph Imaging System, version 5; Universal Imaging Corp.). Areas of the image that were marked with red threshold overlay (as a visual indicator of the threshold areas, in this study GFAP+ astrocytes; Fig. 1C) were included in the measurement and processing (Ramírez JM, et al. *IOVS* 2005;46:ARVO E-Abstract 1318). Individual images were taken with a digital high-resolution camera (CoolSNAP; Photometrics, Tucson, AZ) and were further processed when required (Photoshop CS3 Extended 10.0; Adobe Systems, Inc., San Jose, CA).

**FIGURE 1.** Rat retinal whole mount. (A) Division of the retina in concentric zones for study and areas of retina selected at random from each zone. (B) Photomicrograph of one of the selected areas of the retina. (C) Same area shown in (B) processed with the threshold tool in an imaging system. Red: marked retinal astrocytes that were included in the measurements and processing.





Statistical Analysis

IOP data and AROA among the ipsilateral eyes, the contralateral eyes, and age-matched normal retinas were compared using nonparametric ANOVA with Bonferroni test. A *t*-test was used to compare the IOP between the age-matched control and the contralateral eyes and to compare the AROA, depending on the IOP level. Data are shown as mean ± SD. Differences were considered significant when *P* < 0.05. Pearson correlation was used to analyze the relation between the mean AROA and the mean IOP of each eye.

RESULTS

Age-Matched Control (Naive)

In naive rats, Müller glial cells were undetected for GFAP staining (Figs. 2A–C). GFAP immunoreactivity (GFAP-IR) was localized in stellate astrocytes spaced in a regular fashion in the ganglion cell layer as viewed from the surface (Figs. 2A–C, 3A).

Laser-Induced Ocular Hypertension

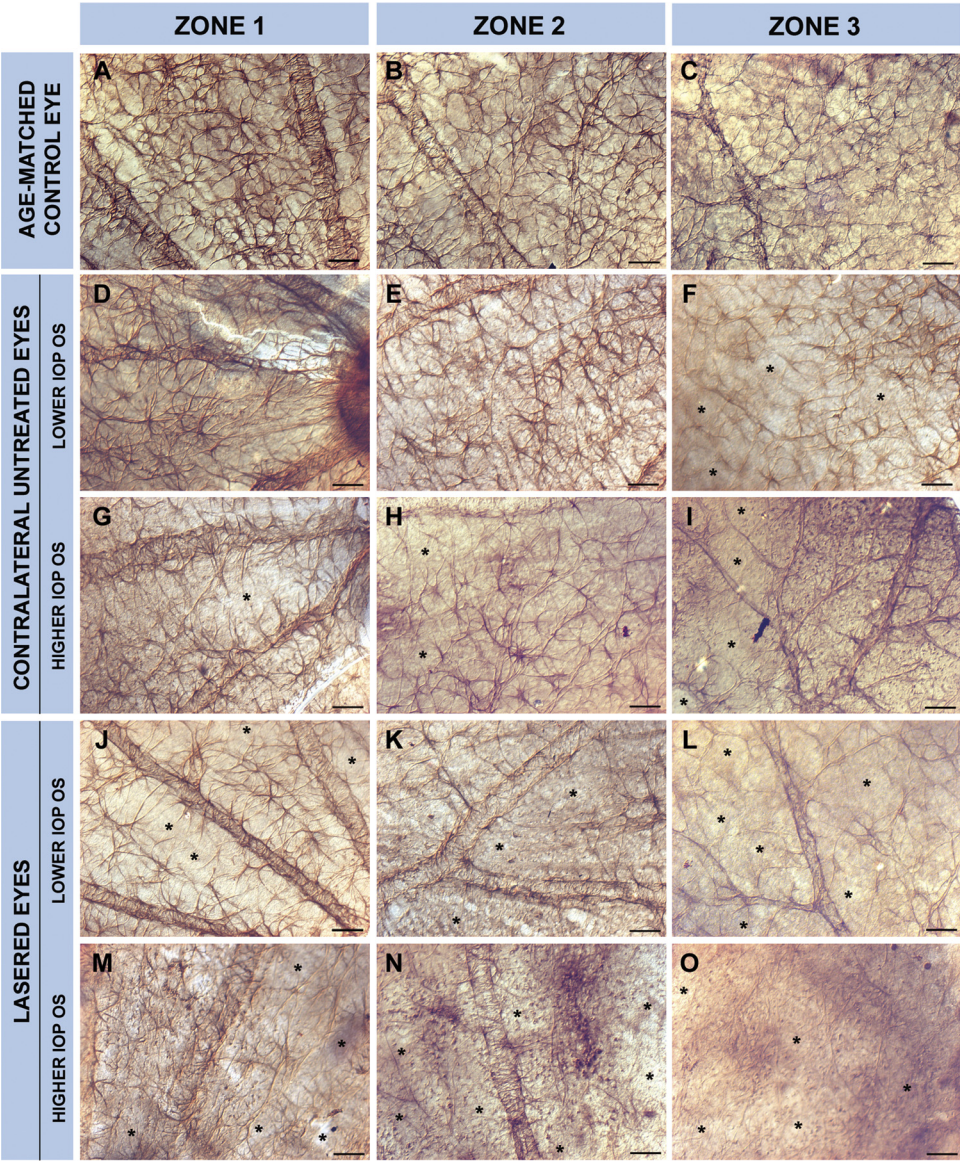
Photocoagulation of the trabecular meshwork and the perilimbal and episcleral veins resulted in a sustained increase in IOP.

There was some variability among the maximum IOP values registered from the lasered eyes within the animals, but overall the results were consistent. IOP values of ipsilateral eyes ( $21.05 \pm 1.73$ ) significantly differed from those of naive ( $16.90 \pm 0.68$ ; *P* < 0.001) and contralateral untreated ( $16.70 \pm 0.76$ ; *P* < 0.001) eyes. No significant differences were found between contralateral and naive.

Effects of OHT in the Retinal Macroglia of the Lasered Eyes

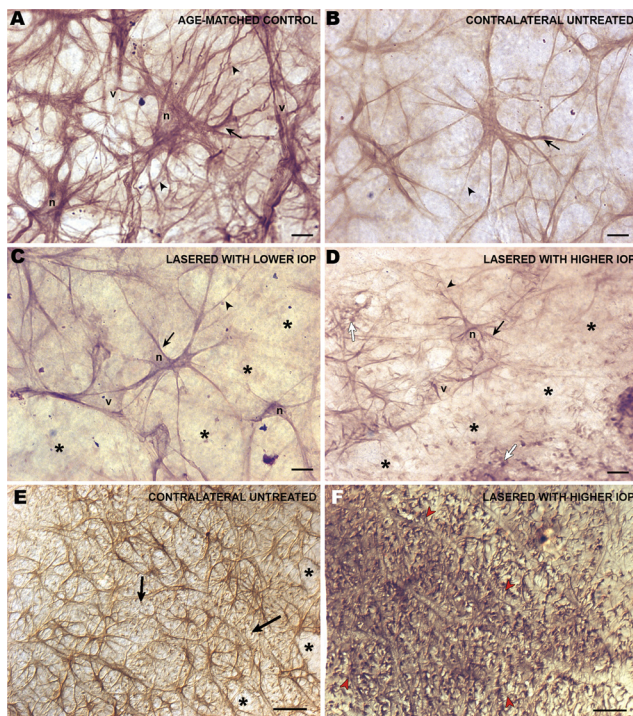
The Müller cells of ipsilateral eyes exhibited GFAP-IR (Figs. 2K, 2M–O, 3D), which appeared as punctate structures between the astrocytes and their radiating processes. This immunostaining varied, depending on IOP values ranging from moderate to intense. In some retinal areas of eyes with higher IOP, Müller cells formed GFAP+ glial scars that precluded astrocyte visualization (Fig. 3F).

No differences in the intensity of astrocyte GFAP-IR of eyes with OHT (Figs. 2J, 2K, 2N) and the contralateral eyes (Figs. 2D, 2E, 2G) were detected in comparison with naive (Fig. 2B) eyes. Overall, astrocytes of the ipsilateral eyes maintained the star-shaped morphology and location similar to those of the



**FIGURE 2.** Comparison by concentric zones chosen for study of the retinal area occupied by astrocytes. Anti-GFAP immunostaining. The intensity of the immunostaining did not differ between the study groups, as observed in the astrocytes located on the vessel wall (A, B, D, E, G, J, K, N). The network of astrocytes was less dense than naive in the three zones analyzed in both ipsilateral and contralateral untreated eyes. In both instances, areas of retina devoid of astrocytes were detected (*asterisks*). The reduction of the area of the retina occupied by astrocytes was greater in lasered eyes (J–O) than in the fellow untreated eyes (D–I). The decrease was more intense for those eyes with higher IOP levels (M–O). Müller cells appeared as GFAP+ punctate structures in both contralateral untreated (E, G, I) and ipsilateral (K, M, N, O) eyes. Contralateral retinas of treated eyes with lower IOP levels (D–F). Contralateral retinas of treated eyes with higher IOP levels (G–H). Zone 1, central; zone 2, intermediate; zone 3, periphery. Scale bars, 50  $\mu$ m. Nomarski optic.





**FIGURE 3.** Morphology of the retinal glia in the different study groups. (A) Astrocytes of age-matched control have a stellate morphology and an oval cell body from which six to eight primary processes (arrow) extended outward and divided into finer secondary ones (arrowhead). (B–D) Astrocytes of ipsilateral and contralateral eyes had fewer secondary processes (arrowhead), and most cell bodies and primary processes (arrow) were thinner than in age-matched control. Müller cells appeared as GFAP+ punctate structures (white arrow). (E, F) GFAP-immunoreaction of Müller cells in a rat with a higher IOP level. (E) GFAP-IR of Müller cells (black arrow) in the contralateral untreated eye. (F) In some retinal areas of the eyes with OHT, Müller cells formed GFAP+ glial scars (red arrowhead) that precluded astrocyte visualization. Such scars were observed neither in naive nor contralateral untreated eyes. n, nucleus; v, blood vessels. Scale bars: (A–D) 10  $\mu$ m; (E, F) 50  $\mu$ m; Nomarski optic.

naive group. However, the astrocytes of ipsilateral eyes had fewer secondary processes, and most cell bodies and primary processes were thinner than in the naive group (Figs. 3C, 3D). Additionally, the astroglial network was less dense and exhibited areas devoid of astrocytes (Figs. 2J–O) compared with the naive group (Figs. 2A–C). Astrocytes in the ipsilateral eyes with lower IOP levels formed a plexus of star-shaped cells in the three zones analyzed (Figs. 2J–L). However, in most samples of ipsilateral eyes with higher IOP levels, the stellate astroglial network could not be recognized in zone 2 (intermediate; Fig. 2N) or zone 3 (periphery; Fig. 2O) primarily because of astrocyte loss.

### Effects of OHT in the Retinal Macroglia of the Contralateral Untreated Eyes

Three weeks after LP, contralateral eyes had macroglial alterations regardless of the IOP value of the ipsilateral eye (Figs. 2D–I). As in eyes with OHT, Müller cells of contralateral eyes differed from naive eyes in exhibiting GFAP-IR (Figs. 2E, 2G, 2I, 3E); however, the glial scars observed in ipsilateral eyes (Figs. 2N, 2O, 3F) were not found in the contralateral untreated eyes (Figs. 2G–I, 3E). Astrocytes of the contralateral eyes formed a plexus of star-shaped cells distributed throughout the retina (Figs. 2D–I), as in naive eyes (Figs. 2A–C). Retinal areas devoid of astrocytes were also detected in the contralateral eyes (Figs.

2F–D), though to a lesser extent than in ipsilateral eyes. As in ipsilateral eyes, astrocytes of contralateral eyes had fewer secondary processes, and most of the cell bodies and primary processes were thinner (Fig. 3B) than in naive eyes (Fig. 3A).

### Area of the Retina Occupied by Astrocytes

In 2 of 14 contralateral retinas, the quality of the immunostaining was inappropriate for AROA quantification.

The AROA of treated eyes showed statistically significant reductions compared with naive ( $P < 0.001$ ) and contralateral untreated ( $P < 0.001$ ) eyes. This feature was observed when the analysis was made both by retinal areas (12 target areas per retina) and by concentric zones of the retina ( $P < 0.001$  for all comparisons) (ANOVA with Bonferroni test). Notably, the AROA in contralateral eyes was statistically significantly reduced compared with naive retinas ( $P < 0.001$ ) (Fig. 4).

Analysis of the AROA in the three groups of eyes studied (naive, ipsilateral, and contralateral) revealed that the AROA in the periphery was significantly reduced compared with the central ( $P < 0.001$ ) and intermediate ( $P < 0.001$ ) zones (ANOVA with Bonferroni test) (Figs. 2, 4).

In OHT, there was a strong correlation ( $r = -0.808$ ;  $P < 0.001$ ) between the mean AROA ( $n = 26$ ) and the mean values of IOP ( $n = 26$ ) of each eye. In contralateral eyes, there was a weak correlation ( $r = 0.296$ ;  $P < 0.351$ ) between mean AROA ( $n = 12$ ) and the mean values of the IOP of the treated eyes ( $n = 12$ ) (Fig. 5).

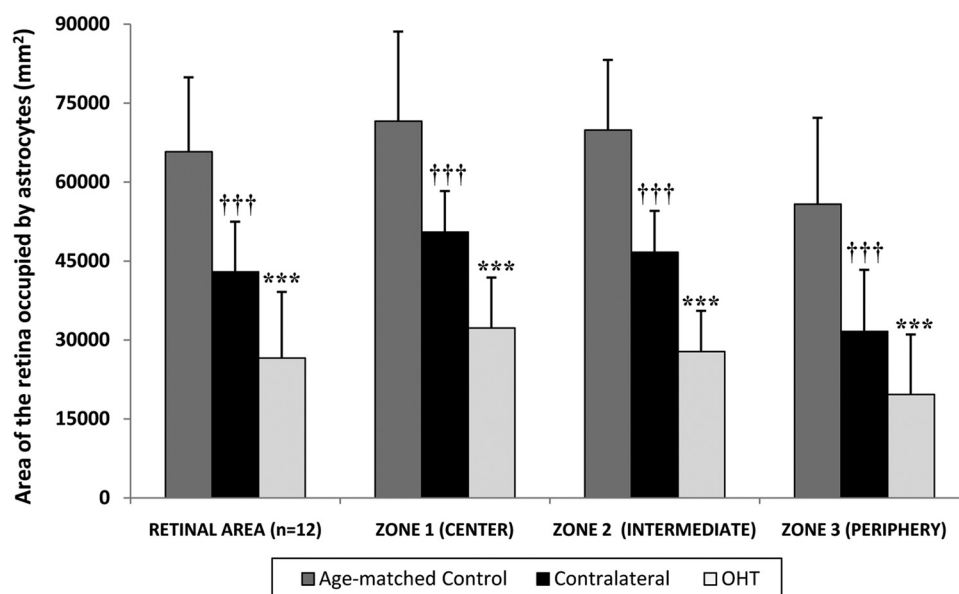
### DISCUSSION

Three weeks after LP, treated eyes experienced significant elevations in IOP compared with contralateral and naive eyes. In parallel studies using a similar methodology, the IOP reportedly increased between 34% and 125% over baseline within the first 72 hours after laser treatment and peaked at 12 hours. At 1 and 2 weeks, the IOP was approximately 35% and 39% over baseline. By 3 weeks the IOP started to decline, acquiring close to normal levels at 12 weeks.<sup>9</sup>

Glial fibrillary acidic protein (GFAP) is a very sensitive marker of glial activation in response to several types of neural insults. The difficulties in distinguishing astrocytes from Müller cells by GFAP immunostaining when using cross-sections are attributed to the fact that astrocytes within the nerve fiber layer express GFAP immunostaining, and Müller cells gain GFAP-IR under a variety of pathologic conditions.<sup>23</sup> The use of flat-mounted preparations of the retina facilitates the differentiation of astrocytes from Müller glial cell end-feet, which otherwise are not readily distinguishable in a sectional profile.<sup>30</sup> In addition, chronically elevated IOP led to the overall increase in the GFAP content of the rat retina, as detected by one-dimensional electrophoresis and immunoblotting despite the reduced GFAP-IR in astrocytes.<sup>23</sup> This fact further underscores the usefulness of retinal flat mounts in evaluating astrocyte reactivity.

Some studies have reported that the aging process increases GFAP-IR in the human brain,<sup>31,32</sup> an observation that has also been reported in the astrocytes of the human retina.<sup>14</sup> Aging does not affect glial activity in the rat optic nerve head until 20 months of age.<sup>33</sup> Thus, in the present study, we used rats at approximately 6 months of age because, at this age, they do not exhibit glial changes compared with retinas of younger animals.

Previous studies using thin sections reported that GFAP-IR and content were increased in human and animal retinas with elevated IOP.<sup>33–36</sup> GFAP-IR of the Müller glia has been reported as early as at the third day after glaucoma induction and persisted for 6 months.<sup>23</sup> In the present study, the intensity



**FIGURE 4.** AROA. Comparison among concentric zones and areas of the retina analyzed in the three study groups. The AROA of treated eyes underwent a statistically significant reduction in comparison with naive ( $P < 0.001$ ) and contralateral untreated ( $P < 0.001$ ) eyes. This feature was observed when the analysis was made both by retinal areas (12 target areas per retina) and by concentric zones of the retina ( $P < 0.001$  for all comparisons). Notably, the AROA in the contralateral untreated eyes showed a statistically significant reduction in comparison with age-matched normal retinas ( $P < 0.001$ ). In the three study groups the peripheral zone had statistically significant less AROA than did the central ( $P < 0.001$ ) and intermediate ( $P < 0.001$ ) zones. Each bar represents the mean  $\pm$  SD of AROA; ANOVA with Bonferroni test. Statistically significant reduction (\*\*\*) compared with age-matched control and contralateral untreated retinas. Statistically significant reduction (†††) compared with age-matched control retinas.

of the GFAP-IR of Müller cells was greater in ipsilateral eyes with higher levels of IOP to such an extent that in some areas, glial scars precluded the visualization of astrocytes. The formation of glial scars in response to OHT has previously been reported.<sup>37,38</sup>

It has been suggested that the postinjury responses of RGCs may elicit a number of glial reactions that have not been completely understood. Retinal astrocytes are able to develop early cellular hypertrophy (because of the upregulation of GFAP+ intermediate filaments, among others) in response to OHT, which increases with time and high IOP.<sup>39</sup> These features have also been found in the astrocytes of the optic nerve.<sup>7,40,41</sup> On the other hand, it has been reported that the GFAP-IR of retinal astrocytes in rats with OHT induced by episcleral vein cauterization is dramatically reduced after 3 days of increased IOP.<sup>23</sup>

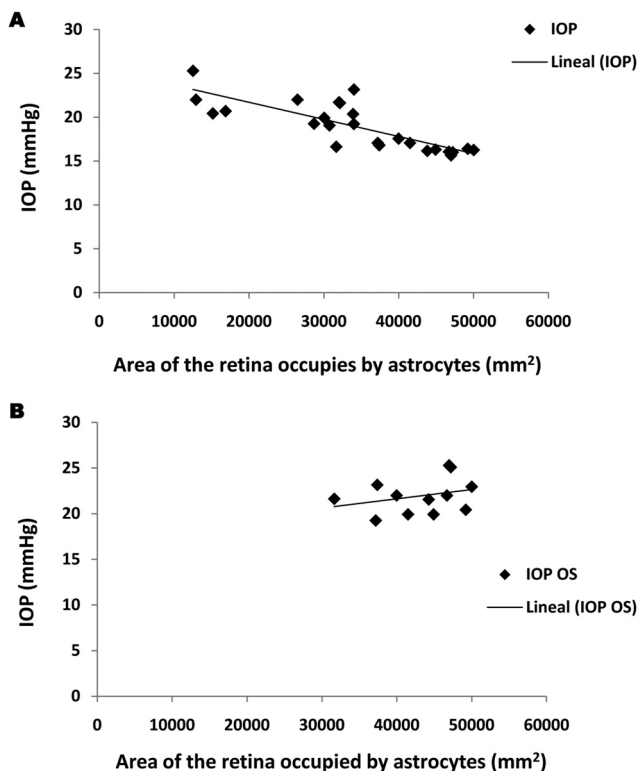
Astrocytes of lasered eyes had thinner cell bodies, fewer secondary processes, and thinner primary processes than those of naive eyes rather than a cellular hypertrophy in response to OHT. Another finding that deserves consideration was that the level of IOP influenced these morphologic changes as well as the amount of AROA lost in such a way that the group with higher levels of IOP had the greater changes. Both facts might explain why, in these retinas, it was difficult to recognize the astroglial network in zones 2 (intermediate) and 3 (periphery).

The retinas of the contralateral untreated eyes had qualitative changes in macroglia similar to those in ipsilateral eyes and significantly fewer astrocytes than in naive eyes. Given that there were no significant differences in IOP values between contralateral and naive eyes, the changes observed in the contralateral retinas could have been driven by the effects of OHT in treated eyes. Kanamori et al.<sup>23</sup> described a gradual change in the GFAP-IR of the Müller cells in the contralateral retina from day 3 after episcleral vein cauterization. Moderate GFAP-IR of the Müller cells has also been reported in the

contralateral eye after optic nerve crush.<sup>42</sup> It has been postulated that bilateral glial proliferation might represent a common acute response to degeneration events both in injured and in contralateral retinas.<sup>43</sup> The glial reactivity observed in the contralateral eye could be related to the potential of the glial cells to initiate, regulate, and sustain an immune response.<sup>44</sup> Similarly, astrocytes of the optic nerve are thought to be capable of mediating immunoreactions, because of their expression of the MHC class II molecule HLA-DR, which is activated in glaucomatous human retina and optic nerve head.<sup>21,45,46</sup> Glial MHC molecules are also upregulated in experimental animal models of glaucoma. It has been postulated that a stimulated T-cell response may be correlated with neuronal damage (Tezel G, et al. *IOVS* 2008;49:ARVO E-Abstract 3699).<sup>22,47</sup> T-cell-derived proinflammatory mediators could act directly on neuronal cells or indirectly by activating local glial cells and attracting and stimulating blood-borne macrophages.<sup>47</sup> It has also been suggested that multiple cell responses in the contralateral eye could be due to the crossing fibers at the optic chiasma or some retinorectal fibers present in rodents.<sup>42</sup> There are other instances of contralateral effects after unilateral tissue damage. It is now well accepted that neurogenic mechanisms contribute to the symmetrical spread of inflammation in rheumatoid arthritis<sup>48,49</sup> and that transneuronal signaling between damaged neurons and their contralateral homologues prevent the spread of peripheral nerve damage.<sup>50</sup> Whether these neurogenic mechanisms are involved in the changes observed in the contralateral untreated eyes in our study deserves further investigation.

We found a strong decreasing linear relationship between IOP and AROA in OHT ( $r = -0.808$ ;  $P < 0.001$ ). Similarly, in primary open-angle glaucoma, the increase in IOP resulted in a progressive loss of RGC axons. In the present experiments, we did not estimate RGC survival, but in a recent parallel study using a comparable methodology to induce OHT, RGC loss was





**FIGURE 5.** IOP versus area of the retina occupied by astrocytes. **(A)** In OHT, there was a strong linear relationship between the mean AROA and the mean values of IOP of each eye. **(B)** In contralateral eyes, there was weak linear relationship between mean AROA and the mean values of the IOP of the treated eyes.

documented using a retrograde tracer applied to both superior colliculi 1 week before animal processing.<sup>9</sup> It is possible that RGC death results from a decrease in glial support, as might be deduced by the activation of the Müller glia and by the reduction of the AROA observed in the present study. Reactive glial cells can exacerbate neuronal damage and may be one of the etiologies of glaucoma through the release of cytokines, reactive oxygen species, and functional disorders of the glutamate uptake in Müller cells.<sup>51,52</sup> On the other hand, we know that astrocytes support damaged neural tissues through the release of neurotrophic factors, antioxidants, and degradation of extracellular deposits.<sup>53</sup> A minimal amount of retina occupied by astrocytes may be necessary to protect RGCs from death; that is, RGC death would begin when astrocytes of the retina diminish beyond a specific level.

Another factor contributing to the impairment of the glial support could be the reduction of astrocyte secondary processes observed in this study. In the human retina, astroglial processes join together by means of desmosomes<sup>15,54</sup> and gap junctions<sup>15</sup> to form a mesh that reinforces the capillary network and supports the neurons in the glial network. These kinds of junctions between processes have also been reported in rats<sup>55</sup> and in other animal species.<sup>56,57</sup> It is known that astrocytes play a decisive role in the metabolism of neurotransmitters and CO<sub>2</sub> and that ions, most sugars, amino acids, nucleotides, vitamins, hormones, and cyclic AMP pass through gap junctions. Apart from coordinating the metabolic activity of cell populations, gap junctions may participate in electrical activities or may amplify the consequences of signal transduction.<sup>58</sup> The reduction of astrocyte secondary processes observed in this study could involve a reduction of their gap junctions and consequently affect neuronal function.

In the present study, contralateral eyes experienced a significant reduction in the AROA compared with naive eyes. These changes in the astroglia appear to take place without a decrease in RGC number. In a recent parallel study focusing on the effect of OHT in the RGCs of adult SD rats, using a comparable methodology to induce OHT, the number of RGCs of the contralateral eye<sup>9</sup> proved similar to that in normal adult SD rats.<sup>59</sup>

In conclusion, here we present novel data regarding the AROA in treated and contralateral untreated eyes in relation to IOP levels. The observation of changes in the astroglia of the contralateral eye led us to conclude that the contralateral eye should not be used as a control eye. The reduction of the retinal area occupied by astrocytes and, consequently, of the glial support provided by these cells could be involved in the diminishing numbers of RGCs reported in eyes with ocular hypertension.

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