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# A Minimally Invasive Experimental Model of Acute Ocular Hypertension with Acute Angle Closure Characteristics

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**Purpose:** To describe a minimally invasive experimental model of acute ocular hypertension (OHT) with characteristics of acute angle closure (AAC).

**Methods:** Adult C57/Bl6 mice (n = 31) were subjected to OHT in one eye using a modified circumlimbal suture technique that elevated intraocular pressure (IOP) for 30 minutes. Contralateral un-operated eyes served as controls. IOP, anterior segment optical coherence tomography, and fundus fluorescein angiography (FFA) were performed. The positive scotopic threshold response (pSTR) and a-wave and b-wave amplitudes were also evaluated. Retinal tissues were immunostained for the retinal ganglion cell (RGC) marker RBPMS and the glial marker GFAP.

**Results:** OHT eyes developed shallower anterior chambers and dilated pupils. FFA showed focal leakage in 32.2% of OHT eyes, but in none of the control eyes. pSTR was significantly reduced at week 1 in OHT eyes compared to control eyes (57.3 $\pm$ 7.2 µV vs. 106.9 $\pm$ 24.8 µV; $P < 0.05$), but a- and b-waves were unaffected. GFAP was upregulated in OHT eyes but not in control eyes or eyes that had been sutured without OHT. RGC density was reduced in OHT eyes after 4 weeks (3857 $\pm$ 143.8) vs. control eyes (4469 $\pm$ 176.0) ($P < 0.05$).

**Conclusions:** Our minimally invasive model resulted in acute OHT with characteristics of AAC in the absence of non-OHT-related neuroinflammatory changes arising from ocular injury alone.

**Translational Relevance:** This model provides a valuable approach to studying specific characteristics of a severe blinding disease in an experimental setting. Focal areas of ischemia were demonstrated, consistent with clinical studies of acute angle closure patients elsewhere, which may indicate the need for further research into how this could affect visual outcome in these patients.

## Introduction

Angle closure glaucoma makes up a significant proportion of cases worldwide, and there is a three-fold higher risk of severe vision losses resulting from this disease as compared to primary open-angle glaucoma.[1–3] The underlying pathogenesis is thought to be multifactorial, although certain anatomical features, such as a shallower anterior chamber or a more convex iris profile, play a prominent role in predisposition toward this disease.[4,5] Several transgenic mouse models of angle closure glaucoma have been described thus far, mostly relating to disrupted anterior

segment genesis.[6–9] These mice typically display iridocorneal adhesions and obliteration of the anterior chamber angle, with a resultant increase in intraocular pressure (IOP) when they are a few months old. However, the utility of these genetically manipulated mouse models is limited, as they require large cohorts of animals and show considerable variability in IOP profile.

The circumlimbal suture method has previously been described as a technique for experimentally inducing ocular hypertension (OHT) in mice.[10,11] Advantages of this model include the preservation of good optical clarity and minimal collateral injury due to the relatively atraumatic nature of the surgery. Other more injurious methods of inducing OHT, such as laser cautery, hypertonic saline injections, or intracameral injections of saline or microbeads to obstruct aqueous outflow, may incite inflammatory changes that are unrelated to the effect of raised IOP. Intracameral cannulation alone, in the absence of IOP elevation, for example, has been shown to increase the density and activity of microglia and macrophages in the retina.[12,13] Therefore, a method of inducing OHT in the absence of these non-specific changes would be desirable to study the effect of raised IOP on the eye. We have modified this method to describe a potential murine model for studying acute ocular hypertension with features of acute angle closure that is highly reproducible and relatively atraumatic and does not require expensive equipment or large numbers of animals to yield consistent results.

Establishing a robust model of angle closure may provide valuable insight into factors that can lead to poorer visual outcomes that are specifically linked to angle closure disease, such as the formation of peripheral anterior synechiae.[4] Applying these methods to transgenic mice could also provide a platform to test how genetic factors that have been associated with angle closure glaucoma in patients, which do not produce a distinct phenotype on their own, can potentially affect molecular changes in the eye in response to experimentally induced angle closure.

# Methods

## Animals and Surgical Technique

Adult male C57/Bl6 mice (n = 31) were bred and treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. This study was approved by the SingHealth Institutional Animal Care and Use Committee (SHS/2017/354). Experimentation commenced at 10 weeks of age. Mice were anesthetized by intraperitoneal injections of 0.1 mL ketamine/xylazine mixture comprised of 2 mg/mL xylazine (Troy Laboratories Pty Ltd, Glendenning, NSW, Australia) and 20 mg/mL ketamine (Ceva Animal Health Pty Ltd, Glenorie, NSW, Australia) for circumlimbal suture application, intraocular pressure measurements, and imaging.

The circumlimbal suture technique was adopted from previous studies,[10,11] where a single 10/0 nylon suture (Ethicon, Inc., Somerville, NJ) was threaded subconjunctivally at four or five evenly spaced points before a knot was tied, approximately aligned with the lateral canthus. The tension on the knot was observed to spontaneously decrease over time if left alone; hence, a 9-mm Barraquer needle holder (Accutome, Inc., Malvern, PA) was further used to clamp the suture between the knot and the sclera, in close apposition with the globe, causing OHT for 30 minutes. Subsequently, the clamp was released and the suture cut. Contralateral un-operated eyes were used as controls in all experiments, except for immunohistochemical evaluation of inflammatory markers where animals underwent circumlimbal suture in both eyes but were only knotted and clamped in one. Sutures were removed from both eyes of these animals after 30 minutes.

## IOP Measurements and Imaging

An Icare TONOLAB rebound tonometer (Icare Finland Oy, Vantaa, Finland) was used to measure IOP at baseline, every 5 minutes while the suture was clamped for up to 30 minutes, and once 5 minutes after the suture was released. IOP was subsequently monitored on day 1 post-procedure (D1), day 3 (D3), and weekly (W1, W2, W3, W4) until animals were sacrificed at week 4. IOP of the contralateral eye was recorded at the same time points. All IOP measurements were taken between 9 AM and 10 AM, with the room lighting at approximately 700 lux; an average of 5 measurements were taken each time per eye by an assessor who was masked to the surgical eye.

Anterior segment optical coherence tomography (AS-OCT) images were obtained (RTvue; Optovue, Inc., Fremont, CA) at baseline, within 5 minutes of suture application (day 0 suture in situ), and immediately following suture removal (day 0 suture cut). AS-OCT images were also taken at D1, W1, W2, and W4 following surgery for measurements of pupil diameter, as well as anterior chamber depth (ACD), where the distance between the corneal endothelium and anterior surface of the iris was recorded.

Fundus imaging and fluorescein angiography were performed following pupil dilation with 1% tropicamide followed by 2.5% phenylephrine (both, Alcon

Laboratories, Inc., Fort Worth, TX). Digital color fundus photographs were taken using the Micron IV retinal imaging microscope (Phoenix Research Laboratories, Pleasanton, CA) centered on the optic nerve head and in all four quadrants. Fundus fluorescein angiography (FFA) was performed after intraperitoneal injections of 1% sodium fluorescein dye (Akorn, Inc., Lake Forest, IL) diluted in sterile saline and given at a dose of 0.1 mL/10 g body weight. FFA images were taken approximately 30 seconds after fluorescein injection and were acquired at baseline, D1, and D3 and then weekly thereafter using an Olympus FV3000 confocal microscope (Olympus Life Science, Waltham, MA) at a magnification of 4×.

Spectral domain OCT images were also taken on the Micron IV (Phoenix Research Laboratories) at baseline, week 2, and week 4. The ganglion cell complex (GCC) thickness, demarcated by the region between the inner limiting membrane and inner nuclear layer, and the total retinal thickness measured up to the retinal pigment epithelium layer were quantified using Image J.

## Electroretinography

Mice were dark adapted for a minimum of 12 hours. All electroretinography (ERG) recordings were performed using the Espion Visual Electrophysiology System (Diagnosys LLC, Westford, MA) in a dark room under dim red light illumination. Mice were anesthetized and their pupils dilated as above. Body temperature was maintained at 37°C using a heat pad fixed on the ERG stage. Vidisic coupling gel (Bausch & Lomb Pharmaceuticals, Inc., Tampa, FL) was applied to each eye, and a gold wire electrode (Grass Technologies, West Warwick, RI) was placed against the corneal surface of both eyes to record electrical signals. A reference electrode (Grass Technologies) was placed in the mouth, and a silver–silver chloride electrode (Grass Technologies) was inserted into the tail as the ground electrode. The mouse head was positioned in front of a Ganzfeld stimulus, and the responses were recorded across different light intensities as follows. Positive scotopic threshold response (pSTR) was defined as the peak amplitude of waveforms recorded at stimulus intensities of –5.22, –4.94, –4.73, and –4.38 log cd·s·m$^2$. The a- and b-wave responses were also recorded at 0.3, 0.7, 1.0, and 1.5 log cd·s·m$^2$. At each intensity, 25 flashes with an interstimulus interval of 3000 ms were averaged. The mean peak or trough amplitudes of each waveform across the different stimulus intensities were calculated. Mean peak pSTR amplitudes across the four different stimulus intensities for OHT or control eyes were further normalized against the b-wave amplitude ratio, (mean b-wave$_{OHT}$)/(mean b-wave$_{control}$), for each animal, based on methods described by Frankfort et al.[14]

## Immunohistochemistry

Mouse eyes were enucleated and processed for retinal flatmounts or cryosections. Retinas were harvested for flatmounts at 4 weeks (n = 16) following experimentally induced angle closure and fixed in 4% paraformaldehyde before staining for the retinal ganglion cell (RGC) marker RBPMS (RNA-binding protein with multiple splicing, GTX118619; GeneTex Inc., Irvine, CA). RGC density was assessed based on images taken from each quadrant (superior, inferior, nasal, temporal) at 20× using an Olympus FV3000 confocal microscope, counted by a masked observer, and divided by the area of retina covered (in mm$^2$). Cryosections were also prepared from optic cups and stained for the glial marker GFAP (glial fibrillary acidic protein, ab7260; Abcam, Cambridge, UK) at 1 week (n = 5), 2 weeks (n = 5), and 4 weeks (n = 5) post-surgery. Anterior segment sections were used for hematoxylin and eosin (H&E) staining.

## Statistical Analysis

Statistical analysis was conducted using Prism 6 software (GraphPad Software, San Diego, CA). Group data are given as mean ± SEM. Results for operated eyes with clamped circumlimbal sutures and raised IOP (OHT) are expressed relative to contralateral control eyes (Control). Tests for normality and homogeneity of variance (Shapiro–Wilk and Bartlett's, respectively) were performed to demonstrate significantly effective pairing before applying paired *t*-tests and repeated measures ANOVA where appropriate.

# Results

## Experimentally Induced Acute Angle Closure Results in Decreased ACD, Increased Pupil Diameter, and OCT

Anterior chamber depth was significantly reduced in OHT eyes as compared to control eyes on the day of surgery (day 0), both with the suture in situ (267.4 ± 20.4 µm vs. control, 377.6 ± 15.0 µm; $P < 0.01$) and after it was removed (276.9 ± 26.2 µm vs. control, 394.6 ± 4.2 µm; $P < 0.01$) (Figs. 1A, 1B). Apart from day 0, there was no significant difference in the ACD of both groups of eyes at any of the other time points (Fig. 1D). H&E staining of anterior

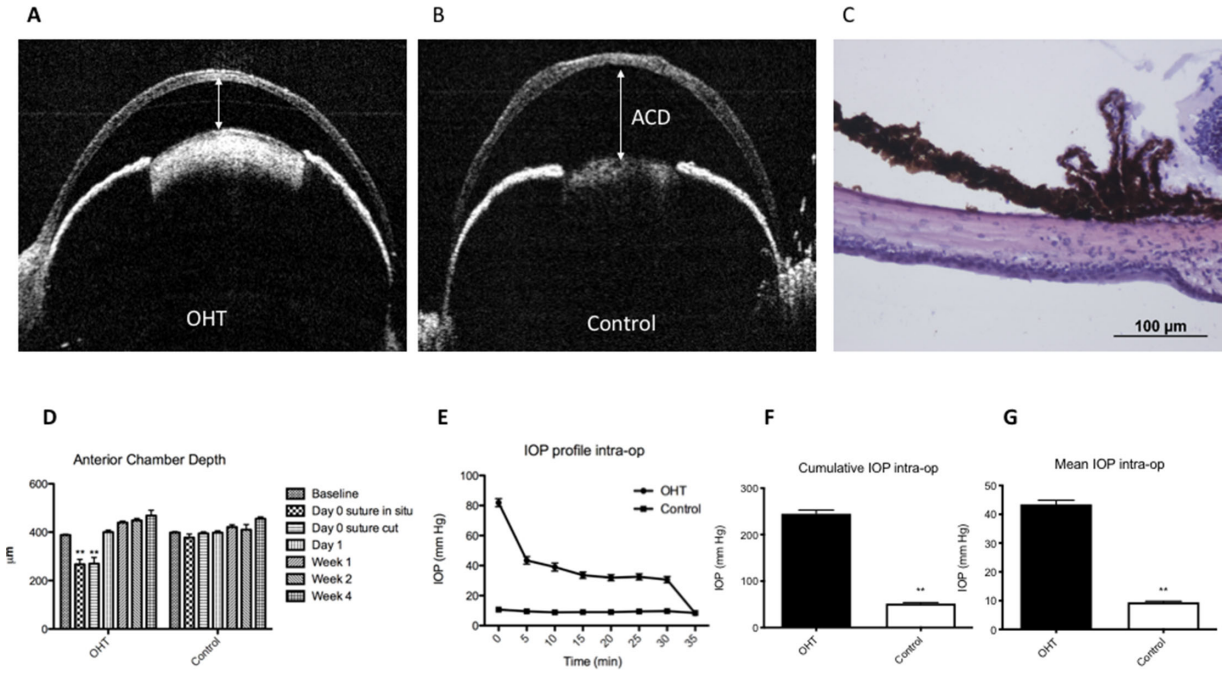


**Figure 1.** Experimentally induced acute angle closure resulted in changes in ACD and IOP on the day of surgery. (A, B) AS-OCT images from OHT and control eyes taken intraoperatively show a reduction in ACD in OHT eyes versus controls, marked by *double-headed arrows*. The iris profile of OHT eyes also appeared steeper, and the pupil diameter was larger in these eyes. (C) H&E staining at week 4 post-induction of acute OHT did not show evidence of peripheral anterior synechiae. (D) ACD measurements were significantly reduced in OHT eyes as compared to control eyes intraoperatively (day 0) with the suture left in situ, but also in measurements taken after the suture was cut at 30 minutes. ACD in OHT versus control eyes did not differ significantly thereafter. (E) Intraoperative IOP measurements showed an initial spike in OHT eyes of up to 80 mm Hg, followed by a gradual reduction until the suture was cut at 30 minutes. (F, G) Cumulative and mean IOP measured intraoperatively with the suture left in situ showed significant increase in the OHT eyes versus controls. $^{**}P < 0.01$; n = 31.

segment sections did not reveal the presence of peripheral anterior synechiae or angle narrowing in OHT eyes (Fig. 1C). Iris profiles in OHT eyes on day 0 also appeared steeper, and the pupil diameter was larger in these eyes compared to controls (874.0 ± 36.4 µm vs. control, 653.7 ± 48.2 µm; $P < 0.01$).

All eyes that underwent circumlimbal suture with clamping for 30 minutes resulted in elevated IOP while the suture remained in situ. OHT eyes briefly experienced a peak IOP of 81.9 ± 2.7 mm Hg versus control (14.2 ± 0.5 mm Hg) at the start of the 30-minute period, but this fell to 43.5 ± 2.5 mm Hg within the first 5 minutes while the clamp and suture were still in place and continued to decrease gradually (Fig. 1D). After removal of the suture, the IOP of OHT eyes was immediately reduced to levels close to those of control eyes (8.2 ± 0.5 mm Hg vs. control, 8.5 ± 0.4 mm Hg). The cumulative IOP of OHT eyes (i.e., the sum of all measurements taken at 5-minute intervals) was 244.5 ± 10.2 mm Hg versus control (51.6 ± 2.6 mm Hg; $P < 0.01$) (Fig. 1F). The mean IOP of OHT eyes over 30 minutes was 43.5 ± 1.8 mm Hg versus control (9.4 ± 0.4 mm Hg; $P < 0.01$) (Fig. 1G).

## Acute OHT Results in a Significant Reduction in RGC Counts and GCC Thickness

Flat-mounted retinas taken from OHT and control eyes 4 weeks (n = 20) after inducing acute angle closure showed a significant reduction in the density of cell nuclei stained with the RGC marker RBPMS in OHT (3857 ± 143.8 cells/mm$^2$) versus control eyes (4469 ± 176.0 cells/mm$^2$) ($P < 0.05$) (Figs. 2A, 2B, 2D). OHT eyes that demonstrated vascular leakage that were evaluated on flatmounts (n = 4) showed a significant reduction in RGC counts (3561 ± 113.2) in comparison with OHT eyes (n = 16) that did not show a significant reduction in RGC counts (4153 ± 53.40) ($P < 0.01$) (Fig. 2E). GCC thickness was also reduced in OHT as compared to control eyes at both 2 weeks (98.72 ± 1.78 µm vs. 104.4 ± 1.67 µm; $P < 0.01$) and 4 weeks (101.6 ± 1.0 µm vs. 105.4 ± 1.26 µm; $P < 0.05$) after experimental acute angle closure (Figs. 2C, 2F, 2G). However, total retinal thickness did not differ between OHT and control eyes at week 2 (261.2 ± 3.0 µm vs. 246.6 ± 6.7 µm) or week 4 (261.7 ± 2.0 µm vs. 256.6 ± 3.2 µm) (Figs. 2H, 2I). There was no significant

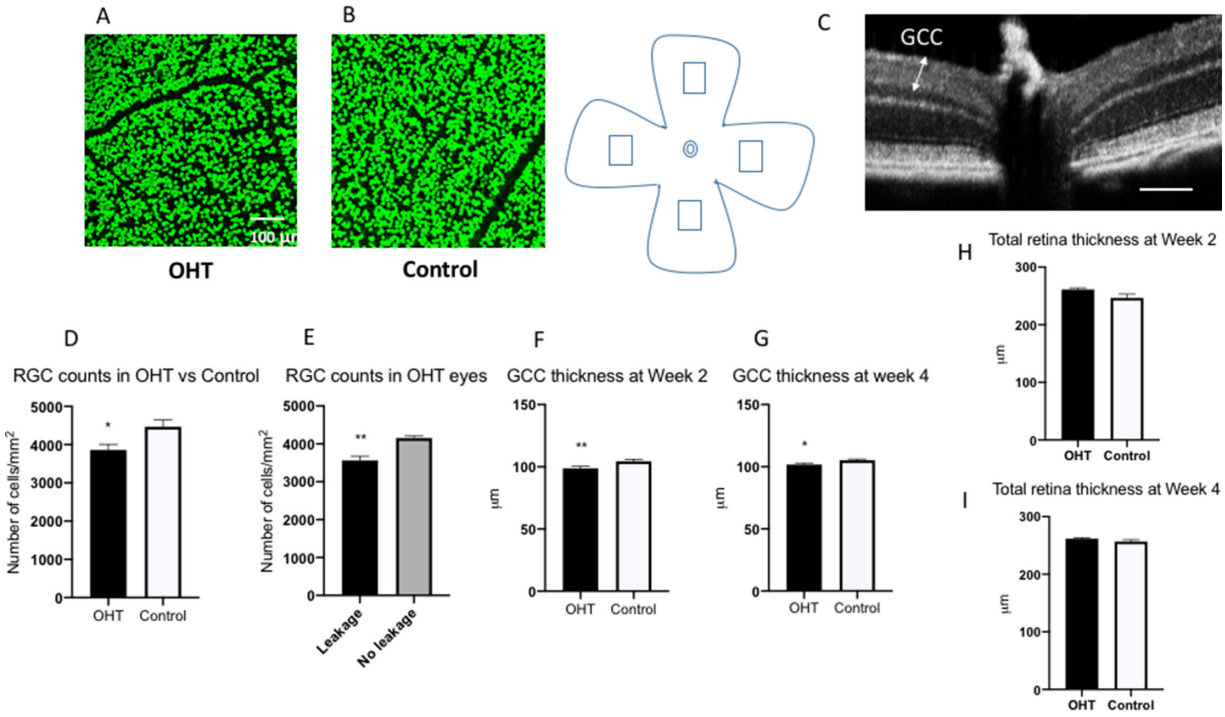


**Figure 2.** Acute angle closure resulted in a significant reduction in RGC counts and GCC thickness at 4 weeks following induction of raised intraocular pressure. (A, B) Representative images of flatmounted retinas from OHT and control eyes, respectively, with the RGC marker RBPMS in *green*. *Scale bar*: 100 µm. (C) GCC thickness was measured from OCT images centered at the optic nerve head, in the area demarcated by *double-headed arrows*. *Scale bar*: 100 µm. (D) RGC counts in flatmounted retinas were significantly reduced in OHT eyes versus controls at 4 weeks following induction of acute angle closure. (E) RGC counts in OHT eyes that showed vascular leakage were significantly reduced as compared to eyes that did not show vascular leakage. (F, G) GCC thickness of OHT versus control eyes was also reduced at both 2 and 4 weeks following induction of acute angle closure. (H, I) Total retinal thickness at 2 and 4 weeks did not differ significantly between OHT and control eyes. $^{*}P < 0.05$, $^{**}P < 0.01$. Sample size at week 2, n = 26; week 4, n = 2.

difference in the RGC counts among each quadrant of the retina, comparing OHT and control eyes (inferior: 1508 ± 425 cells/mm$^2$ vs. 1760 ± 250 cells/mm$^2$, $P = 0.71$; nasal: 1235 ± 262 cells/mm$^2$ vs. 2006 ± 61 cells/mm$^2$, $P = 0.61$; superior: 1220 ± 253 cells/mm$^2$ vs. 2097 ± 36 cells/mm$^2$, $P = 0.57$; temporal: 1505 ± 427 cells/mm$^2$ vs. 1773 ± 86 cells/mm$^2$, $P = 0.33$).

## OHT Eyes Demonstrate Early Loss of Inner Retinal Electrophysiological Response but Outer Retinal Responses Are Preserved

Electrophysiological studies of OHT eyes versus control eyes showed a significant decrease in normalized pSTR amplitude at 1 week (n = 31) following experimental acute angle closure (57.3 ± 7.2 µV vs. 106.9 ± 24.8 µV; $P < 0.05$), suggesting inner retinal dysfunction. In the following weeks, however, the pSTR in OHT eyes gradually increased again in weeks 2 (n = 26) and 4 (n = 21) but did not recover to levels similar to those in control eyes (week 2: OHT 73.6 ± 5.9 µV vs. control 108.1 ± 20.7 µV, $P = 0.59$; week 4: OHT 75.6 ± 20.9 µV vs. control 91.8 ± 25.3 µV, $P = 0.45$). The a- and b-wave responses in both OHT and control eyes did not differ significantly at any of the time points studied (Fig. 3).

## Acute Angle Closure Results in Focal Leakage of Retinal Capillaries in Some OHT Eyes, which was not Seen in Controls

High-resolution FFA demonstrated clear leakage in about one-third of eyes that experienced OHT with acute angle closure (10 out of 31 mice). These areas of leakage occurred from smaller vessels (arterioles, venules, or capillaries) and generally resolved after 1 week. Areas of leakage were only faintly detectable with lower resolution imaging. Colored fundus photographs showed regions of retinal pallor corresponding to areas of leakage, which also diminished over time (Figs. 4A–4F). The presence of small

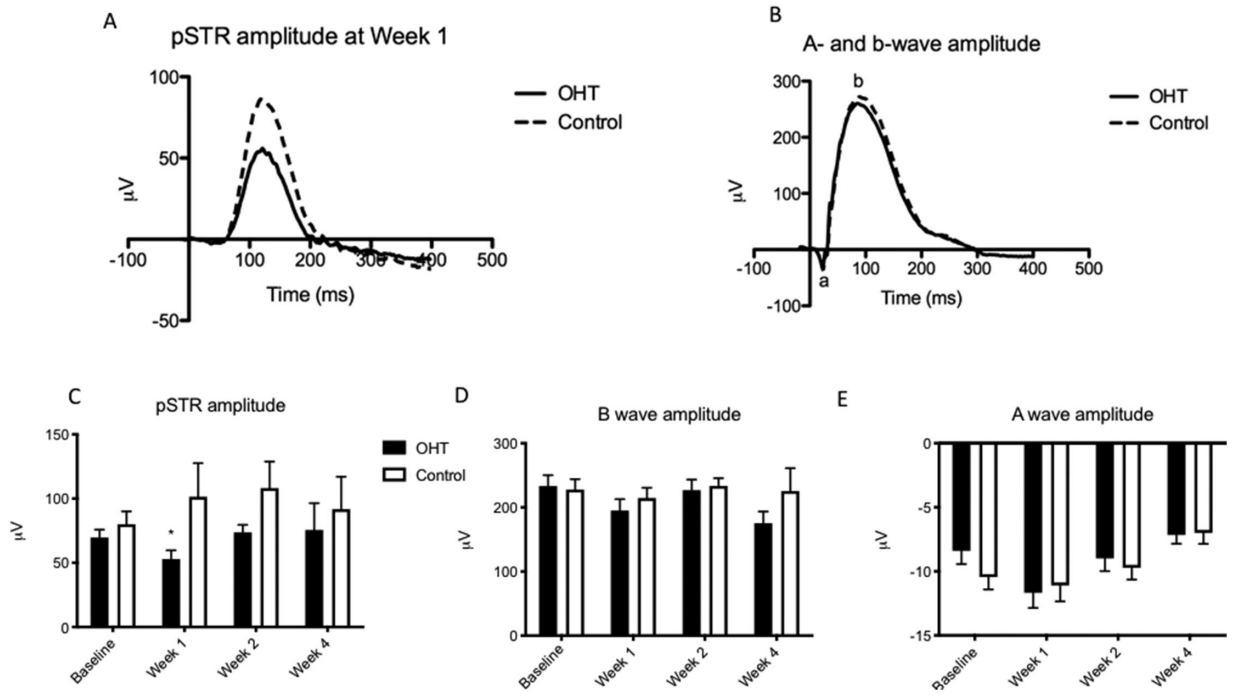


**Figure 3.** OHT eyes demonstrated early loss of inner retinal electrophysiological response, but outer retinal responses were preserved. (A, B) Representative pSTR amplitudes and a- and b-wave responses, respectively, in OHT and control eyes. (C) pSTR amplitudes in OHT eyes were significantly reduced at week 1 but subsequently increased again relative to control eyes. (D, E) The b- and a-wave amplitudes, respectively, do not demonstrate any significant difference in OHT versus control eyes at any time point. $^{*}P < 0.05$. Sample size at baseline, n = 31; week 1, n = 31; week 2, n = 26; week 4, n = 21.

blood vessel leakage did not appear to be associated with higher IOP or increased glial reactivity. OHT eyes showed variable evidence of both low GFAP (Fig. 4G) and increased GFAP (Fig. 4H) immunoreactivity, which also was not associated with IOP levels. OHT eyes that showed vascular leakage did not differ from eyes that did not show signs of leakage on FFA in terms of IOP profile, GCC thickness, pSTR, or GFAP expression (Supplementary Fig.), although there was notably reduced RGC density in eyes with leakage as compared to those without, as mentioned earlier.

Contralateral eyes that had undergone circumlimbal suture without knotting and clamping did not demonstrate evidence of increased immunoreactivity for GFAP at week 1 (similar to Fig. 4G). Therefore, control eyes were subsequently not subjected to circumlimbal suture.

## Discussion

The present study demonstrates short-term IOP elevation and RGC loss with accompanying transient inner retinal dysfunction using a minimally invasive technique, which displays features of acute angle closure such as reduced ACD and increased pupil diameter. Eyes which had been subjected to circumlimbal suture alone in the absence of OHT did not demonstrate a significant neuroinflammatory response.

We did not measure the mean arterial pressure in our study, although sedation of adult C57/Bl6 mice under similar anesthetic regimes has been reported to result in a pronounced drop in arterial pressure, where the average systolic blood pressure and mean arterial pressure were 112 ± 10 mm Hg and 80 ± 10 mm Hg, respectively.[15] Consistent with these findings, studies examining mouse models of ischemia–reperfusion injury using intracameral cannulation describe IOP levels that are typically higher (120 mmHg) and elevated for a longer duration (60[16] to 90 minutes[17]) than what was observed in our experiments. Prolonged exposure to such high IOP was associated with significant impairment of the outer retinal ERG response in these studies. In rats, however, research has suggested that increasing IOP to above 50 mm Hg for more than 105 minutes in an intracameral cannulation model is sufficient to cause outer retina dysfunction, consistent with ischemic injury.[18,19] The mean peak IOP in eyes that had undergone experimentally induced acute angle

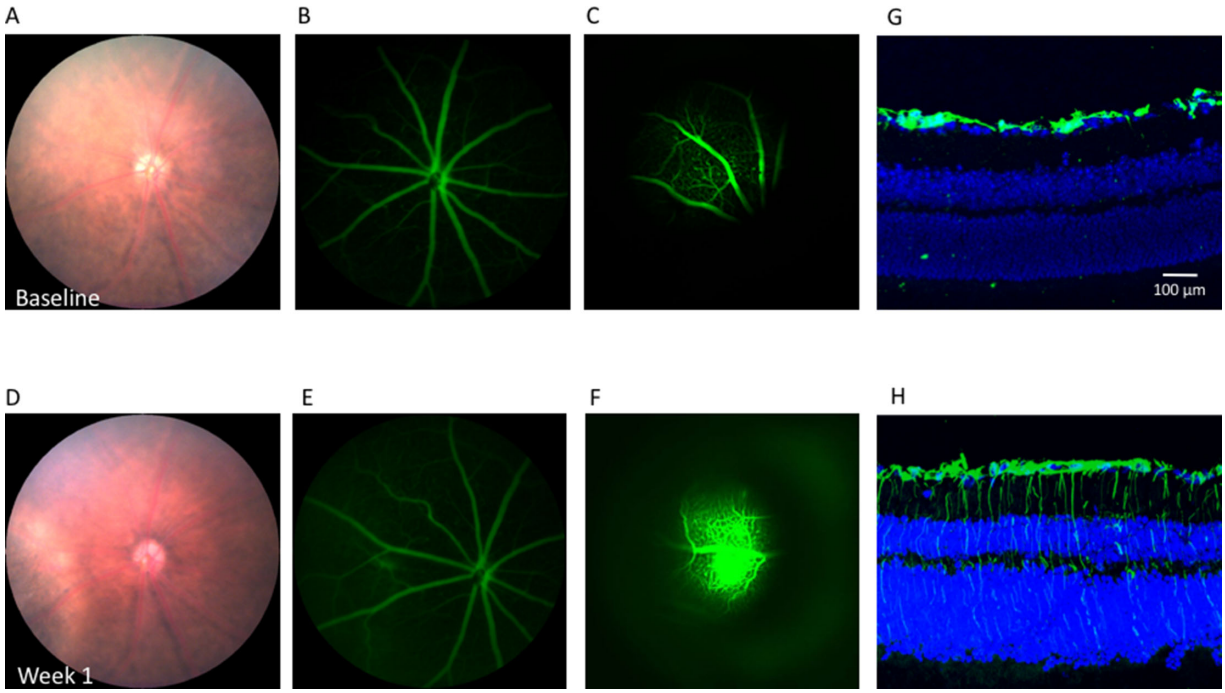


**Figure 4.** Acute angle closure resulted in focal leakage of retinal capillaries in some OHT eyes, which was not seen in controls. (A–C) Baseline fundal photographs, low-resolution FFA, and high-resolution FFA, respectively, for comparison. Fundus photograph at week 1 (D) shows a focal area of hypopigmentation, with associated capillary leakage on low-resolution FFA (E) and high-resolution FFA (F). Gross vessel morphology and retinal perfusion looked otherwise intact. (G, H) Retinal sections from OHT eyes showed variable evidence of GFAP (*green*) immunostaining at week 1. Contralateral eyes that had been sutured without knotting and did not experience OHT had an appearance similar to that of G.

closure in our study was 82 mm Hg for up to 5 minutes, which may have resulted in a period of decreased perfusion and retinal ischemia, although there did not appear to be any lasting detrimental effect on outer retinal function based on our ERG findings.

Approximately one-third of the eyes that experienced acute angle closure with elevated IOP in our model also showed signs of leakage from the small blood vessels in the retina with high-resolution FFA. These regions corresponded with areas of retinal pallor seen on colored fundus photography, which may represent discrete spots of ischemia. Furthermore, greater RGC loss experienced by OHT eyes that showed signs of leakage as compared to those that did not could imply more severe injury arising from ischemia, although their IOP profiles did not significantly differ.

Patients with acute primary angle closure (APAC) typically present with extremely high IOP of between 50 and 60 mm Hg[20] in comparison with primary open-angle glaucoma. Signs of focal ischemia in the eye, such as iris atrophy and glaucomflecken, have also been reported in up to half of all APAC patients.[21] A clinical study of long-term outcomes of patients following an APAC attack has revealed that 20% of cases had optic disc pallor out of proportion to glaucomatous cupping, similarly implying that ischemic changes had occurred.[22] It is important to note that evidence of prolonged retinal ischemia or ischemic sequelae such as ocular neovascularization has not been described in acute angle closure patients, despite such patients presenting with IOP measurements that exceed central retinal artery perfusion pressure.[23] We did not observe ocular neovascularization in our mouse model of angle closure, either, although this may be related to the rapid re-establishment of blood flow following 30 minutes of IOP elevation. Nonetheless, our discovery of focal areas of ischemia in our model could indicate a greater need to study the effect of raised IOP on retinal circulation in APAC patients, as well.[24]

The mean IOP in eyes that had undergone experimentally induced acute angle closure was 43.5 mm Hg over 30 minutes in our study. Similar levels of OHT have been achieved by intracameral cannulation with a glass microcapillary attached to a saline reservoir.[13,23] Kezic et al.[13] found that mice that experienced IOP elevation to 50 mm Hg for 30 minutes with this procedure showed depressed electrophysiological responses that were specific to the inner retina at 7 days, which is consistent with our findings. Interestingly, our results suggest that, at later time points following the

initial IOP insult, there may be partial recovery of inner retinal function, although RGC counts and GCC thickness measures indicated a loss of RGC at 4 weeks following acute IOP elevation. This could indicate that the remaining RGCs were able to partially compensate for the functional loss that was incurred just after the initial insult. Both experimental mouse studies and clinical research have suggested that measures to reduce intraocular pressure may result in improvements in visual function in glaucoma.[25–28] This is an intriguing prospect that would certainly warrant further investigation.

A prominent advantage of our acute angle closure model is that the surgical procedure is confined to the subconjunctival space, with no ocular penetration and minimal trauma. The inflammatory marker GFAP was not shown to be significantly upregulated in eyes that had been subjected to circumlimbal suture in the absence of IOP elevation or acute angle closure. Therefore, the resultant effects on RGC structure and function in OHT eyes are likely to have arisen directly from IOP elevation secondary to angle closure in keeping with the clinical pathogenesis of acute primary angle closure in patients.

Experimental mice that had experienced IOP elevation in our model also maintained sufficient optical clarity to enable high-resolution imaging of the retinal vasculature, which showed small retinal blood vessel leakage in some animals, although there was no correlation with IOP levels. Apart from optic disc hemorrhages, increased vessel permeability has not previously been reported in association with glaucoma or raised IOP. Pharmacological disruption of the blood–neural barrier has been observed to occur earlier in retinal vessels than in the cortical vasculature,[29] which may indicate greater vulnerability of the blood–retinal barrier in the eye. Differential genetic expression of several factors that mediate vascular permeability such as caveolin 1 and 2 have been found to be associated with glaucoma.[30] The nitric oxide–guanylate cyclase pathway has also been widely implicated in the pathology of glaucoma and is involved in the regulation of vascular permeability.[31] Soluble guanylate cyclases (sGCs), in particular, are expressed in RGCs, photoreceptors, trabecular meshwork cells, and the vascular smooth muscle layer of retinal arterioles,[32] and they play a key anti-inflammatory role.[33] sGC knockout mice demonstrate thinning of the retinal nerve fiber layer and loss of optic nerve axons in the presence of a very modest 2-mm Hg increase in IOP; it is possible that disruption of the endothelial barrier may further contribute to RGC injury in glaucoma.

Limitations of our study include the transient nature of the IOP elevation, making it unsuitable for studying a chronic disease such as primary angle closure glaucoma. Continuous IOP monitoring during placement of the circumlimbal suture would have helped to determine if there were further IOP spikes beyond what was measured at 5-minute intervals, although this was not possible due to the limitations of our setup. We were also unable to accurately quantify the spatial or temporal characteristics of the vascular leakage or to determine the retinal layer in which it was occurring. Further studies with OCT angiography would no doubt be of value to investigate potential areas of ischemia more extensively. It is also possible that the resultant OHT in operated eyes was partially caused by distal outflow obstruction, as it was difficult to ensure that the episcleral veins were not compressed by the circumlimbal suture due to the small dimensions of the mouse eye, although every effort was made to avoid this. Another important structural limitation of our experimental model is the lack of a distinct scleral spur on AS-OCT images of the mouse eye, which made it difficult to quantify the extent of iridotrabecular contact or angle narrowing. Other groups have reported successful visualization of anterior chamber angle structures using a special goniolens,[34] which was not available for our study. The interstimulus interval and stimulus intensities used for recording bright flash responses could have been increased further to avoid issues with b-wave contamination or light adaption; these settings will be adjusted for future experiments. Studying the effect of acute OHT with decreased ACD on GCC thickness or RGC counts at the earlier time point of week 1 would also provide further insight into how this injury affects RGCs, in addition to the ERG findings presented. Finally, we did not measure eye length in our experiments because of the lack of equipment available in our animal facility; hence, we cannot exclude the possibility that elongation of the eye ball associated with the pronounced increase in IOP may result in retinal damage.

Epidemiological studies consistently show that patients suffering from primary angle closure glaucoma (PACG) have a higher risk of experiencing severe visual impairment in comparison with primary open-angle glaucoma and possess anatomical risk factors for angle closure disease such as short axial length, shallow anterior chamber, thick lens, thick iris, and anterior lens position.[1–3] Recent literature has also suggested that significant differences in iris and choroidal volume can be found in PACG patients, as compared to a normal population.[5] These ocular features that predispose angle closure glaucoma are difficult to study in an experimental setting. A single report based on a *N*-ethyl-*N*-nitrosurea mutagenesis screen described a specific mutation (glaucoma-related mutation 4, *Grm4*)

on serine protease Prss56 that resulted in reduced axial length and anterior chamber depth and narrow angles in adult mice.[35] Many mouse models that describe an "angle closure" phenotype are based on disrupted anterior segment development that does not represent the majority of angle closure disease seen in patients. Furthermore, the effects of intermittent angle closure on the iris, trabecular meshwork, retinal and choroid vasculature, and optic nerve remain largely unknown. It is possible that prevention of angle closure sequelae or understanding the downstream effects of this condition could have a significant impact on reducing rates of blindness caused by glaucoma. Consequently, our experimental model, which is easily reproducible and demonstrates the main features of acute angle closure, could further aid our understanding of this important disease.

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