# Effect of Anterior Chamber Cannulation and Acute IOP Elevation on Retinal Macrophages in the Adult Mouse

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**Purpose.** To assess the retinal macrophage response to cannulation of the anterior chamber (AC) and acute elevation of intraocular pressure (IOP) in adult mice.

**Methods.** Eyes from 12-month-old C57BL/6J WT mice were subject to IOP increase (50 mm Hg for 30 minutes) by direct cannulation of the AC. Fellow eyes were either cannulated without pressure increase or left untreated. Electroretinography was carried out prior to IOP elevation and 1 week later. Immunofluorescence staining was performed on frozen sections and retinal wholemounts 1 week after sham and IOP elevation. Eyes were assessed by epifluorescence and confocal microscopy and the density of vitreal hyalocytes and subretinal macrophages was calculated.

**Results.** The density of hyalocytes and subretinal macrophages was significantly increased 1 week after IOP elevation and AC cannulation compared with naïve eyes. CD68 and MHC Class II expression was upregulated in both cannulated eyes and eyes with elevated IOP. Electroretinographic signals derived from retinal ganglion cells were significantly reduced in response to acute IOP elevation, but not in response to cannulation alone.

**Conclusions.** Cannulation of the AC causes an increase in hyalocyte density, microglial activation, and accumulation of macrophages in the subretinal space. These macrophage changes are similar to those observed in eyes subject to IOP elevation. Additional IOP elevation led to significant Müller cell activation, which was not evident after cannulation alone. These data highlight the importance of using appropriate controls in models of acute retinal injury.

Keywords: macrophage, retina, microglia, IOP

Glaucoma is a group of multifactorial, optic neuropathies, which involve a slow progressive degeneration of retinal ganglion cells and their axons.[1,2] Other than aging, one of the major risk factors for glaucoma is elevated intraocular pressure (IOP) and thus, rodent models for glaucoma research have largely been centered around targeting elevated IOP for potential therapeutic applications.[3,4] Whilst the underlying pathophysiology of glaucoma remains unclear, there is accumulating evidence that an immune or parainflammatory component may contribute to disease pathogenesis,[5–12] with particular interest in the contribution of retinal microglia to disease progression.[13–16]

Microglia are a specialized population of resident macrophages in central nervous system parenchyma, being well recognized for their performance of surveillance, scavenging and homeostatic functions associated with host defense and tissue repair.[17–19] Indeed, the phagocytosis of dying or degenerating neurons by microglia has been well established,[20–23] and more recent studies demonstrate a prominent role for microglia in the process of synaptic pruning in the postnatal mouse brain.[24,25] Whilst primed microglia can exert beneficial effects essential to neuronal survival and neurogenesis through the release of various trophic and anti-inflammatory factors,[26] activated microglia have the ability to generate reactive oxygen species, increase phagocytic activity, and produce inflammatory cytokines including tumor necrosis factor alpha (TNFα); interleukin-6 (IL-6); IL-1β; and IL-12p40.[27] The morphological transformation of microglia from a highly ramified form to an amoeboid shape is commonly used as a measure of activation,[26,28–30] in addition to the upregulated expression of immune markers including MHC Class II, CD68 and Isolectin-b4 (IB4).[26,31] Accordingly, retinal microglia have been the subject of investigation in numerous ocular disease models, including rodent models of autoimmune uveitis,[32,33] light-induced photoreceptor damage,[34,35] diabetic retinopathy,[36] and glaucoma.[14,15,37]

In human glaucoma, microglia are present at initial sites of axonal degeneration, indicated by abnormal reactivity and distribution of these cells at the optic nerve head.[38,39] Similarly, in rodent models of glaucoma, microglial activation appears to be the earliest detectable change in the retina,[15] with the activation of retinal microglia coinciding with retinal ganglion cell degeneration.[37] It is thus plausible that microglia are key contributors to a pathogenic inflammatory milieu during the progression of disease and may consequently present a potential link to retinal ganglion cell survival.

Given the interest in the potential role of microglia in retinal ganglion cell injury, we sought to determine whether deficits in

retinal function following the acute elevation of IOP correlated with alterations to retinal microglia. To assess the retinal macrophage response to acute IOP elevation in the adult mouse eye, we used an established model whereby the AC is cannulated and IOP elevated to 50 mm Hg for 30 minutes. This induces a highly repeatable nonlethal injury that causes oxidative stress in the inner retina and loss of the inner retinal derived positive scotopic threshold response (pSTR), whilst maintaining outer retinal function as measured using electroretinography. Full functional recovery of the pSTR occurs in young (3-month) but not older mice (18-month).[40] Given that the susceptibility of retinal ganglion cell damage appears to increase with age,[41,42] this acute model of retinal injury is a valuable tool to assess the way in which ocular responses to injury or stress change during normal aging.

In the present study, to ensure any changes observed in microglia were specific to pressure elevation rather than in response to injury alone, we included both naïve fellow eyes and fellow eyes that received cannulation of the AC alone (without pressure elevation) as controls. The data revealed a marked retinal macrophage response to acute IOP elevation as well as following cannulation of the AC without pressure elevation, with similar increases in vitreal hyalocyte and subretinal macrophage densities, as well as upregulated expression of the immune markers MHC Class II, CD68, and Iba-1. Interestingly, despite these macrophage responses being apparent in both cannulated eyes and eyes exposed to IOP elevation, retinal function was only impaired in IOP elevated eyes, suggesting different mechanisms are involved in injury alone versus pressure elevation. This is the first study to fully describe retinal macrophage changes in response to a nonischemic, acute elevation of IOP, as well as in response to cannulation alone. These findings highlight the importance of using appropriate controls in studies of elevated IOP whereby an injury is part of the experimental procedure.

## Methods

### Animals

C57BL/6J mice aged 12 months were bred and housed at the Royal Victorian Eye and Ear Hospital (RVEEH) and were maintained in a 22°C, 12-hour light (~40 lux)/12-hour dark environment with standard murine chow (WEHI breeder mix; Barastoc, VIC, Australia). Food and water were supplied ad libitum. All experimental and animal care procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the RVEEH Animal Ethics Committee.

### Electroretinography

The full-field flash ERG was used to assess retinal function in dark-adapted (overnight >12 hours) anesthetized (intraperitoneal injection of 70 mg/kg ketamine, 7 mg/kg xylazine; Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) animals using established protocols.[43] Pupils were dilated with 0.5% tropicamide (Alcon Laboratories Pty Ltd., Frenchs Forest, NSW, Australia) and 2.5% phenylephrine (Minims; Chauvin Pharmaceuticals, Surrey, UK). Signals were recorded with a 4-mm platinum wire electrode contacting the cornea while a gold pellet placed in the mouth served as reference. A subdermal needle inserted in the tail acted as ground. Retinal responses to a series of stimulus intensities (–5.92–2.22 log cd.s/$m^2$) were recorded simultaneously from both eyes over a period of 30 minutes. For data analysis, maximum amplitudes of the photoreceptor-derived a-wave and bipolar cell-derived b-wave were measured in response to a flash stimulus of 2.22 log cd.s/$m^2$. Amplitudes of the positive scotopic threshold response (pSTR) were taken at a fixed time of 110 ms after a flash stimulus of –4.54 log cd.s/$m^2$, which coincides with the pSTR peak in control responses. ERGs were recorded serially in animals, 1 day before (baseline) and 7 days after IOP elevation.

### Acute Elevation of IOP

The acute elevation of IOP was performed on anesthetized mice as previously described.[40] Briefly, the AC of one eye was cannulated with a borosilicate glass needle (~50 μm, 1B100-6; WPI, Sarasota, FL) connected to a pressure transducer (Transpac IV; Abbot Critical Care Systems, Sligo, Ireland) via polyethylene tubing (0.97 mm inner diameter; Microtube Extrusions, North Rocks, NWS, Australia), which was in series with a sterile Hanks' balanced salt solution reservoir (sterile-filtered, endotoxin tested; catalogue #H6648; Sigma-Aldrich, St. Louis, MO). IOP was raised by altering the height of the reservoir and monitored in real time using a PowerLab data acquisition system (ADInstruments, Colorado Springs, CO). For acute elevation of IOP, pressure was adjusted to 50 mm Hg for 30 minutes. Control eyes were cannulated and pressure was maintained at physiological IOP (12 mm Hg). Resting IOP of all eyes, measured immediately prior to cannulation using a handheld rebound tonometer, was 11.57 ± 0.21.

### Tissue Collection and Processing

For immunofluorescence staining of retinal wholemounts, mice were euthanized by intraperitoneal injection with sodium pentobarbitone (100 mg/kg; Virbac, Milperra, NSW, Australia) and fixed by intracardial perfusion with 4% paraformaldehyde (PFA) as previously described.[44] For frozen sectioning, eyes were enucleated and immersion-fixed in 4% PFA for 3 hours, followed by overnight cryoprotection in 15% sucrose. Eyes were embedded in optical cutting temperature (OCT) medium and 10-μm sections cut through the papillary-optic nerve axis at different levels of the globe.

### Immunofluorescence Staining of Tissue Sections and Retinal Wholemounts

Retinal and choroidal wholemounts were prepared as previously documented.[33] Briefly, tissues were incubated in 20 mM EDTA tetrasodium (37°C) for 30 minutes and then blocked for 60 minutes at room temperature (RT) with 3% bovine serum albumin and 0.3% Triton-X solution in PBS. Tissues were treated with one of the following antibodies: polyclonal rabbit anti-Iba-1 (ionized calcium binding adaptor molecule 1; Wako Pure Chemical Industries Ltd., Osaka, Japan); biotinylated isolectin-B4 (Vector Laboratories, Burlingame, CA); mouse anti-GFAP (glial fibrillary acidic protein; BD Pharmingen, Inc., San Diego, CA); rat anti mouse CD68 (scavenger receptor; AbD Serotec, Raleigh, NC); and rat anti mouse MHC Class II (M5/114; BD Pharmingen, Inc.) at 4°C overnight. Samples were then incubated with biotin-conjugated anti-rat (1:300; Amersham Biosciences, Piscataway, NJ); biotin-conjugated anti-rabbit (1:300, Vector Laboratories) antibody or anti-mouse Alexa Fluor 488 (1:400; Molecular Probes, Eugene, OR) for 2 hours at RT. Samples treated with biotinylated antibodies were incubated for 2 hours (RT) with Steptavidin-conjugated Cy3 (Jackson ImmunoResearch, West Grove, PA). Hoescht was used for nuclear staining. Retinae were mounted on slides with the vitreous side up. Immunostained wholemounts were imaged using epifluorescence (Olympus Provis AX70 microscope with AnalySIS software; Olympus Soft Imaging Solutions, Münster, Germany) and confocal microscopy (Nikon C1 Upright; Nikon

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Australia Pty Ltd., Lidcombe, NSW, Australia). Confocal microscopic images spanning the full thickness of retinal tissue were prepared by scanning at 1-μm increments (×20 objective lens). To calculate the density of hyalocytes, six epifluorescence images from randomly selected areas of anti-Iba-1 stained retinal tissues were taken at the level of the nerve fiber layer (NFL) and Iba-1$^+$ hyalocytes counted by a masked observer. To calculate the density of macrophages in the subretinal space, Iba-1$^+$ cells in the photoreceptor cell layer (PCL) were counted in retinal wholemounts and combined with counts of Iba-1$^+$ cells on the retinal pigment epithelial surface of choroidal wholemounts from the same region. Final image processing was performed using a graphics editing program (Adobe Photoshop version 7.0; Adobe Systems, Inc., San Jose, CA).

### Statistical Analysis

All data were analyzed using unpaired Student's *t*-test ($P < 0.05$ considered as significant). Data are presented as mean ± standard error of the mean (SEM).

## Results

### Retinal Microglial Responses to AC Cannulation and Acute IOP Elevation

Adult (12-month-old) C57BL/6J mice were subject to cannulation alone, whereby the AC was cannulated and the IOP maintained at physiological levels (12 mm Hg; $n = 4$), or the IOP was elevated to 50 mm Hg for 30 minutes ($n = 9$). The fellow eye of some IOP elevated eyes were not cannulated, to assess whether a contralateral eye effect was evident ($n = 6$). Eyes were collected 1 week later and processed for immunofluorescence staining of retinal wholemounts. The 1-week time point was selected as prior work had shown no changes in macrophages 24 hours after cannulation or IOP elevation (data not shown). Comparisons of confocal microscopic images of Iba-1$^+$ retinal microglia in the outer plexiform layer (OPL) revealed only slight disruption to the regularly spaced microglial network in cannulated or IOP elevated eyes when compared with eyes from naïve mice (Figs. 1A–C). Assessment at higher magnification revealed that a subpopulation of OPL microglia in cannulated and IOP elevated eyes had more rounded, elongated cell bodies when compared with microglia in naïve eyes (Figs. 1D–F). Iba-1 and CD68 expression were upregulated in IOP elevated eyes when compared with expression in the naïve retina and cannulated control eyes (Figs. 1G–I). The analysis of whole retinal scans viewed in side (*z*) profile demonstrated the extension of microglial processes toward the subretinal space in cannulated and IOP elevated eyes, as well as a greater cluster of Iba-1$^+$ and CD68$^+$ cells in the NFL of IOP elevated eyes (Figs. 1G–I).

### Iba-1$^+$ Vitreal Hyalocytes Accumulate 1 Week After Cannulation of the AC and IOP Elevation

Hyalocytes are resident macrophages of the vitreous body, being in close proximity to the inner limiting membrane of the retina. These cells appear to be early responders to inflammatory events, with increased cell density of vitreal hyalocytes having been reported following TLR9 mediated retinal inflammation, as well as in background retinopathy (*Ins2*$^{Akita}$ mice) and VEGF-driven retinal pathology (*Kimba* mice).[45,46] To determine whether hyalocyte number is altered in response to acute elevation of IOP, cell densities of Iba-1$^+$ hyalocytes were calculated and compared between groups 1 week after treatment. Interestingly, hyalocyte numbers were similarly increased in both cannulated and IOP elevated eyes, when compared with 12-month-old naïve eyes (Fig. 2A). To assess activation of vitreal hyalocytes, the percentage of rounded versus pleiomorphic Iba-1$^+$ hyalocytes at the NFL was calculated in naïve (fellow eye of treated mice), cannulated, and IOP elevated eyes. Whilst there were no Iba-1$^+$ hyalocytes of rounded morphology in 12-month-old naïve eyes, there was a significantly higher percentage of round hyalocytes in IOP elevated eyes when compared with cannulated eyes ($P = 0.025$, Fig. 2B). Immunofluorescence staining of retinal wholemounts revealed upregulation of CD68 expression on hyalocytes in both cannulated (Fig. 2D) and IOP elevated eyes (Fig. 2E) when compared with naïve eyes (Fig. 2C). As expected, MHC Class II was expressed on perivascular macrophages in naïve eyes (Fig. 2F). However, the expression of MHC Class II was upregulated on vitreal hyalocytes in both cannulated eyes (Fig. 2G; 16.5 MHC Class II$^+$ hyalocytes/mm$^2$, 30% of Iba-1$^+$ hyalocytes) and IOP elevated eyes (Fig. 2H; 24 MHC Class II$^+$ hyalocytes/mm$^2$, 37% of Iba-1$^+$ hyalocytes), indicative of activation of these cells. Confirmation of hyalocyte activation was demonstrated by the expression of Isolectin-B4 (IB4) on these cells in cannulated (Fig. 2J; 6.36 IB4$^+$ hyalocytes per mm$^2$, 16% of Iba-1$^+$ hyalocytes) and IOP elevated eyes (Fig. 2K; 16.25 IB4$^+$ hyalocytes per mm$^2$, 30% of Iba-1$^+$ hyalocytes), whereas IB4 was only present on the retinal vasculature in naïve eyes (Fig. 2I).

### Accumulation of Macrophages in the Subretinal Space 1 Week After Cannulation of the AC and IOP Elevation

It is well recognized that with advancing age, microglia migrate to and accumulate in the subretinal space (between the retinal photoreceptors and retinal pigment epithelium).[47–49] Subretinal macrophage accumulation is also generally considered a hallmark of disease in the retina, having been shown in response to light-induced damage to photoreceptors,[34,35,50] as well as in murine models of experimental autoimmune uveoretinitis (EAU)[33] and background diabetic retinopathy.[46] Here, the density of subretinal macrophages was calculated in naïve (noncannulated) eyes of 12-month-old mice (fellow eye of treated mice), as well as 1 week after AC cannulation or elevation of IOP (Fig. 3A). Microglia accumulated in the subretinal space following AC cannulation (Figs. 3A, 3B) and acute IOP elevation (Figs. 3A, 3C). Iba-1$^+$ subretinal cells were evident in the photoreceptor cell layer of retinal wholemounts (Figs. 3D–F), as well as on the RPE surface of choroidal wholemount preparations (Figs. 3G–I). These data demonstrate that cannulation of the AC (injury alone) without pressure increase results in a marked macrophage response in the retina.

### Müller Cell Activation Following Acute Elevation of IOP but Not AC Cannulation

To determine whether macroglial changes occurred in the retina following AC cannulation and acute elevation of IOP, GFAP expression was analyzed in both retinal wholemounts (Figs. 4A–C) and frozen tissue sections (Figs. 4D–F). In 12-month-old naïve eyes, we noted normal expression of GFAP on astrocytes in the NFL (Fig. 4A) of retinal wholemounts. This was also the case in cannulated eyes (Fig. 4B); however, the distribution of GFAP expression was disrupted in IOP elevated eyes (Fig. 4C). These data were confirmed with immunofluorescence staining of retinal sections, with normal GFAP expression evident in both naïve (not shown) and cannulated

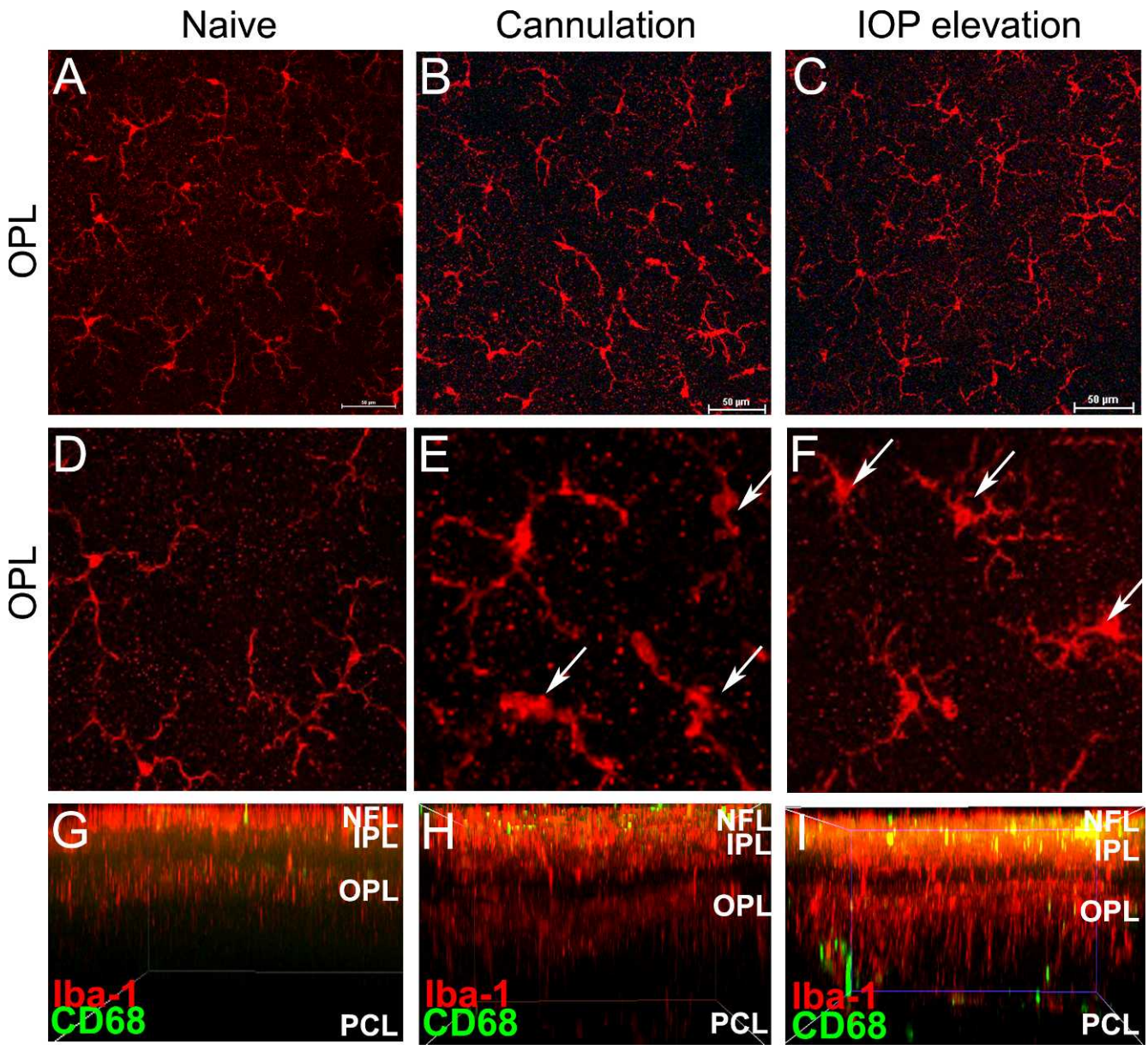


**Figure 1.** Confocal microscopic analysis of Iba-1 stained retinal wholemounts from 12-month-old C57BL/6J naïve (**A**, **D**, **G**) mice and 1 week after AC cannulation (**B**, **E**, **H**) or acute elevation of IOP (**C**, **F**, **I**). (**A**–**C**) Distribution of Iba-1$^+$ microglia in the OPL of the naïve mouse (**A**) and after cannulation (**B**) or acute elevation of IOP (**C**). Disruption of the microglial network was evident in cannulated (**B**) and IOP elevated (**C**) eyes. (**D**–**F**) Higher magnification images of OPL microglia showing normal ramified morphology in the naïve mouse (**D**). Note the more rounded, elongated soma in cannulated (**E**) and IOP elevated (**F**) eyes. (**G**–**I**) Whole retina confocal scans as viewed in side profile showing Iba-1 and CD68 expression in the naïve mouse (**G**) and 1 week after cannulation (**H**) or acute elevation of IOP (**I**). Note increased expression of both Iba-1 and CD68 in eyes that had been subject to IOP elevation (**I**) when compared with naïve and cannulated eyes. IPL, inner plexiform layer.

eyes (Fig. 4D), and the redistribution or upregulation of GFAP expression on Müller cells in IOP elevated eyes (Fig. 4E).

## Retinal Function Decreases in Response to Acute IOP

To test the effect of AC cannulation and IOP elevation on retinal function, we used the full-field flash ERG. Three measures of ERG amplitude were taken, reflecting contributions from different retinal neuronal types: the photoreceptor-derived a-wave, the bipolar cell-derived b-wave, and the positive scotopic threshold response (pSTR). The pSTR in mice largely reflects retinal ganglion cell activity and has been shown to be sensitive to both acute and chronic IOP insults.[40,51,52] ERGs were recorded serially from animals prior to (baseline) and 7 days after IOP elevation, and results are presented as relative changes in amplitude from baseline (Fig. 5).

In response to IOP elevation, amplitudes of the pSTR component of the ERG were reduced sharply to 54.4% of baseline values, consistent with previous reports.[40] This reduction was significantly different ($P = 0.005$) from the response seen in cannulated contralateral eyes, in which amplitudes were maintained at 92.5% of initial values. Neither AC cannulation alone nor acute elevation of IOP had a significant effect on ERG signals derived from outer and middle retina. Amplitudes of the photoreceptor-derived a-wave

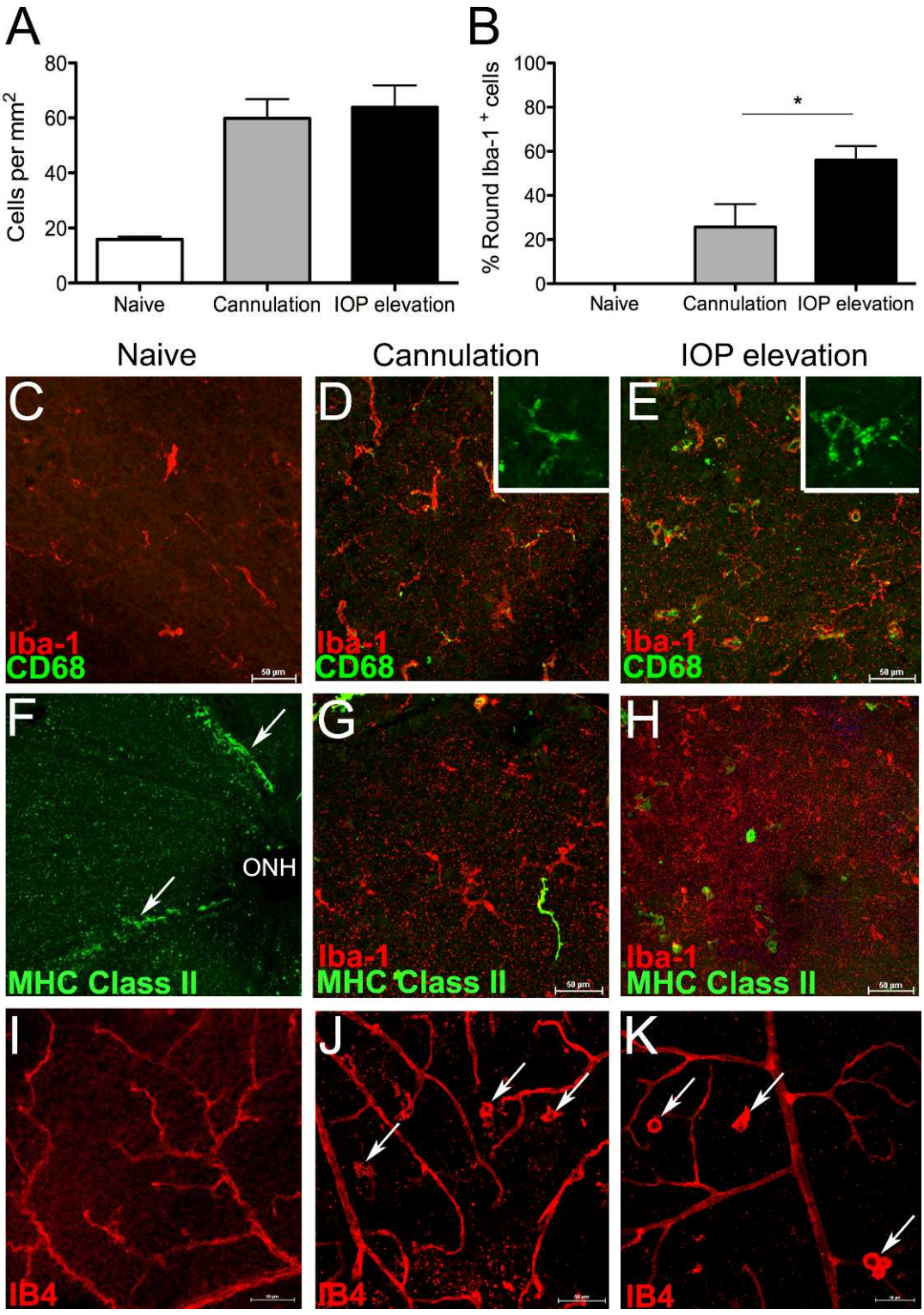


FIGURE 2. Mean density of Iba-1$^+$ vitreal hyalocytes in naïve mice and 1 week after cannulation or acute elevation of IOP ([**A**]; $n = 6$ naïve, $n = 4$ cannulation, $n = 9$ IOP elevation). *Error bars* indicate SEM. Percentage rounded Iba-1$^+$ hyalocytes in naïve mice and 1 week after AC cannulation or acute elevation of intraocular pressure (**B**). $^*P < 0.05$. (**C**–**E**) Confocal microscopic analysis of Iba-1 and CD68 expression on hyalocytes at the level of the NFL in retinal wholemounts from naïve mice (**C**) and 1 week after cannulation (**D**) or elevation of IOP (**E**). Note increased expression of CD68 on hyalocytes in both cannulation ([**D**], *inset*) and IOP elevation groups ([**E**], *inset*). MHC Class II was expressed on perivascular macrophages in the naïve mouse eye ([**F**], *arrows*). MHC Class II expression was upregulated on hyalocytes in cannulated (**G**) and IOP elevated eyes (**H**). IB4 was expressed on the inner retinal vessels in naïve eyes (**I**). IB4 expression was upregulated on hyalocytes in cannulated ([**J**], *arrows*) and IOP elevation groups ([**K**], *arrows*), indicative of activation. ONH, optic nerve head.

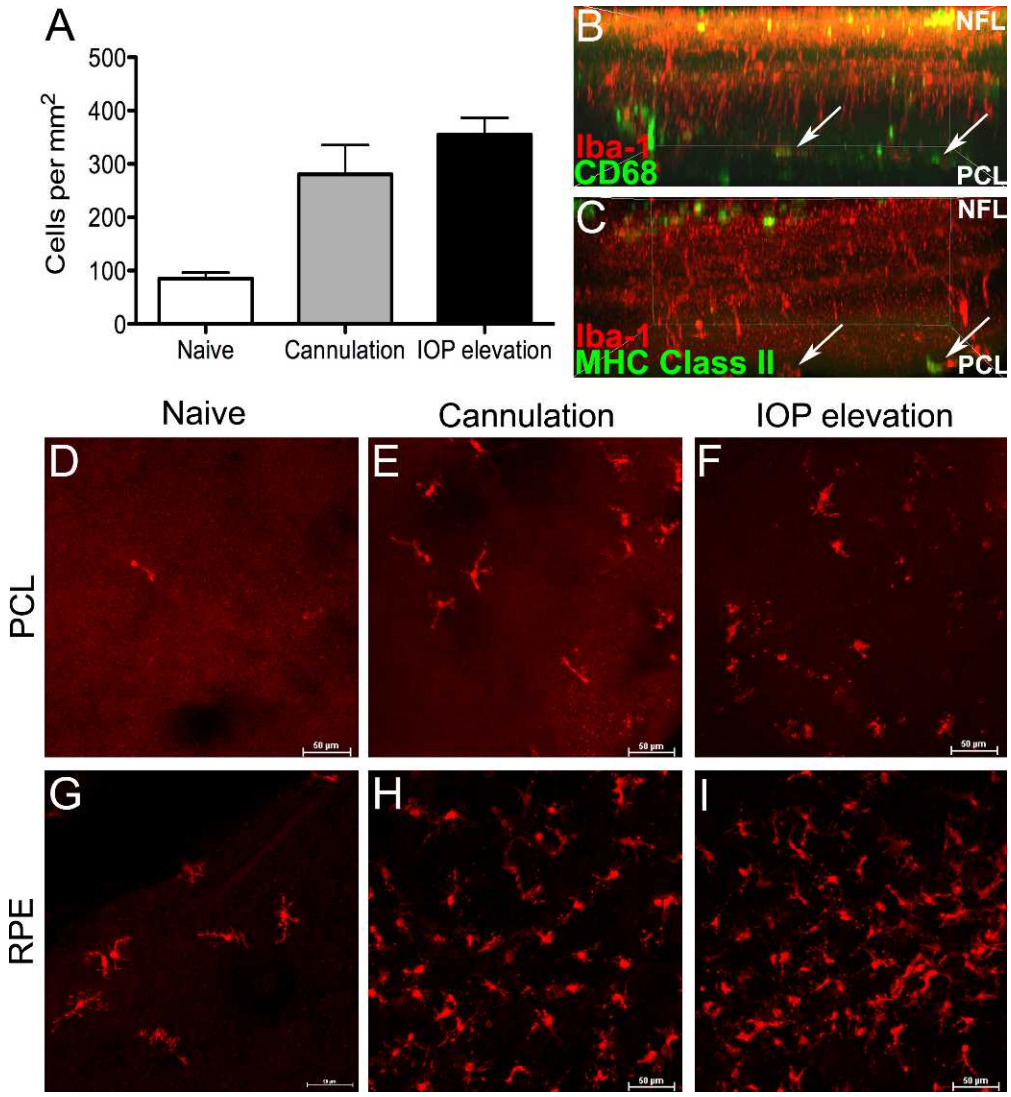


FIGURE 3. Mean density of Iba-1$^+$ microglia in the subretinal space in naïve mice and 1 week after cannulation or acute elevation of IOP ([**A**]; $n = 6$ naïve, $n = 4$ cannulation, $n = 9$ IOP elevation). *Error bars* indicate SEM. Confocal microscopic analysis of whole retina confocal scan as viewed in side profile, showing expression of Iba-1 (**B**, **C**); CD68 ([**B**], *arrows*; cannulation); and MHC Class II ([**C**], *arrows*; IOP elevation) on microglia in the subretinal space. (**D**–**F**) Confocal microscopic images of retinal wholemounts at the level of the PCL showing Iba-1$^+$ microglia in naïve mice (**D**) and 1 week after cannulation (**E**) or elevation of IOP (**F**). (**G**–**I**) Iba-1$^+$ microglia on the surface of the RPE in choroidal wholemounts from naïve mice (**G**) and 1 week after cannulation (**H**) or IOP elevation (**I**).

and bipolar cell-derived b-wave in IOP-elevated eyes were maintained at 84.4% and 89.5% of baseline values, and were not significantly different from amplitude changes seen in fellow cannulated eyes (reductions to 83.3% and 84.9%).

## DISCUSSION

A prominent role for the immune system and specifically, immunocompetent cells such as retinal microglia in the pathogenesis of glaucoma has emerged, with both studies in rodent models of the disease and those examining human tissue demonstrating microglial activation as the earliest detectable change in the retina,[15] as well as the presence of microglia at initial sites of axonal degeneration.[38,39] In the present study, we report a marked macrophage response to both AC cannulation (without pressure elevation) and acute elevation of IOP, as demonstrated by increases in vitreal hyalocyte and subretinal macrophage densities, and the upregulation of the immune markers MHC Class II, CD68, and Iba-1 on retinal macrophages 1 week after injury. Surprisingly, cannulation of the AC alone also produced substantial changes to retinal macrophages, which were similar to those observed in eyes that had been exposed to acute IOP elevation. Acute IOP elevation induced additional upregulation of GFAP on Müller cells, which was not evident in cannulated eyes not subject to IOP elevation. Additionally, although we detected similar retinal macrophage responses in both cannulated eyes and eyes with elevated IOP, inner retinal function was only impaired in IOP elevated eyes, suggesting that macrophage responses to pressure elevation may not be directly related to neuronal function.

The acute model of retinal injury used in the present study, whereby IOP is elevated to 50 mm Hg for 30 minutes, is a valuable experimental tool to assess the ability of the retina and optic nerve to recover from a highly reproducible acute injury. We have carefully characterized this model in prior reports.[40] Previous studies in our laboratory have found that this duration

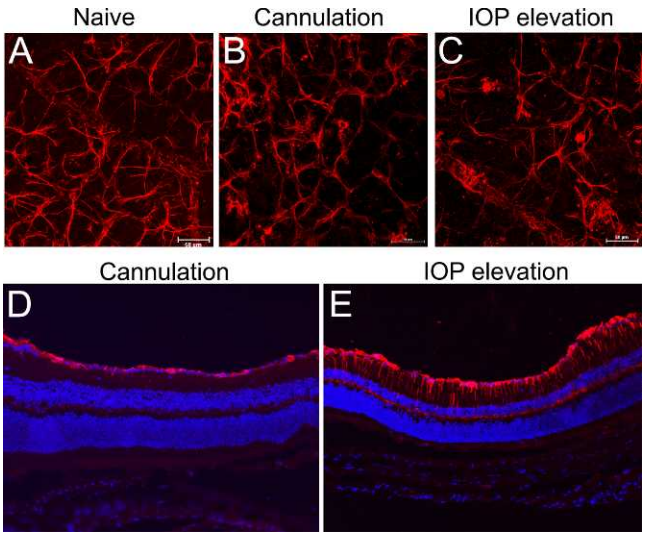


FIGURE 4. Confocal microscopic analysis of GFAP expression on astrocytes in retinal wholemounts in naïve mice (**A**) and 1 week after cannulation (**B**) or elevation of IOP (**C**). The regular distribution of $GFAP^+$ astrocytes in the NFL was disrupted 1 week after IOP elevation (**C**). GFAP expression in retinal sections 1 week after cannulation alone (**D**) or elevation of IOP (**E**). Note upregulation of GFAP on Müller cells in IOP elevated eyes only (**E**).

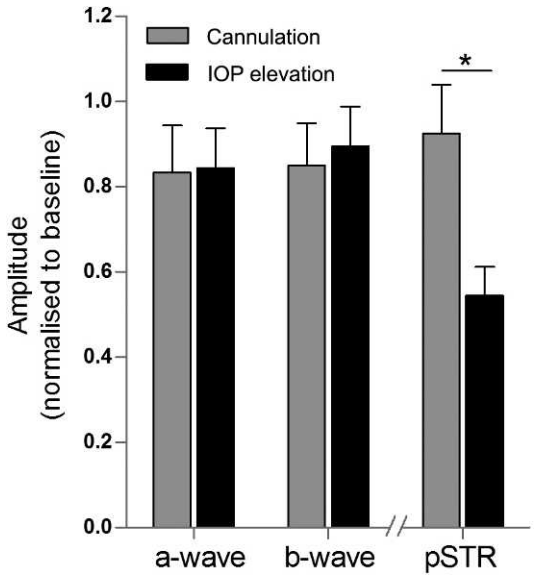


FIGURE 5. Electroretinographic analysis of retinal function in 12-month-old C57BL/6J mice after cannulation and acute elevation of IOP. Electrical responses originating from photoreceptors (a-wave); bipolar cells (b-wave); and retinal ganglion cells (pSTR) were recorded serially from individual animals, before and 1 week after cannulation and data are presented as relative changes form baseline. Amplitudes were measured in response to flash stimuli of 2.22 log cd.s/$m^2$ (a-wave and b-wave) and −4.54 log cd.s/$m^2$ (pSTR). Data are presented as mean and SEM for $n = 12$ per group. $^*P < 0.05$.

and level of pressure produces maximum functional impairment of the inner retina (retinal ganglion cells) while maintaining outer retinal function.[40] Additionally, this level of pressure produces consistent oxidative stress without the induction of ischemia, as indicated by low hypoxia-inducible factor-1 alpha (HIF-1α) levels, and there is minimal (<5% loss) loss of retinal ganglion cells 1 week after IOP elevation (unpublished observations). Thus, the changes to retinal macrophages observed in the present study following both AC cannulation alone and acute elevation of IOP appear to be early indicators of stress in the retina.

Previous studies using sham cannulation as a control in fellow eyes of those subject to acute elevation of IOP have not reported microglial changes in response to injury alone.[53] Additionally, little is known about the contralateral eye response to acute IOP elevation. In studies using the laser-induced ocular hypertension model, Gallego et al. recently reported microglial reactivity and upregulation of MHC Class II on microglia in the naïve eye contralateral to the lasered eye.[16] Although in the present study we report marked changes to retinal macrophages in response to AC cannulation of control eyes, we did not detect a contralateral effect in naïve fellow eyes 1 week after IOP elevation. These contradictory findings further highlight the different mechanisms involved in varying experimental models of elevated IOP. Further assessment of microglial responses to IOP elevation and the correlation of these responses to impairments in inner retinal function in experimental rodent models will lead to a better understanding of why retinal ganglion cells are predisposed to injury in the aging eye, and how microglial responses may contribute to this injury.

The present study characterized retinal macrophage changes to the nonischemic, acute elevation of IOP, and demonstrated similar retinal macrophage responses occur after cannulation of the AC alone. The accumulation of vitreal hyalocytes and subretinal macrophages, as well as microglial activation, occurred 1 week after injury in both cannulated eyes and eyes that had been exposed to IOP elevation, despite functional deficits and Müller cell activation evident only in the IOP elevation group. These data suggest that the retinal macrophage changes observed in response to acute IOP elevation may not be directly related to the functional changes observed. As such, further studies are required to determine the significance of these macrophage responses, and to assess whether or not activated macrophages contribute to changes in retinal function in this injury model. The marked macrophage response following cannulation alone in the present study highlights the importance of using appropriate controls in models of acute retinal injury.

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