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REVIEW

Potential mechanisms of retinal ganglion cell type-specific vulnerability in glaucoma

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[Running head]
Retinal ganglion cells in glaucoma *Wang, Lee, Bui et al.*

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[Abstract]

Glaucoma is a neurodegenerative disease characterised by progressive damage to the retinal ganglion cells (RGCs), the output neurons of the retina. RGCs are a heterogenous class of retinal neurons which can be classified into multiple types based on morphological, functional and genetic characteristics. This review examines the body of evidence supporting type-specific vulnerability of RGCs in glaucoma and explores potential mechanisms by which this might come about. Studies of donor tissue from glaucoma patients have generally noted greater vulnerability of larger RGC types. Models of glaucoma induced in primate, cat and mouse also show selective effects on RGC types –particularly OFF RGCs. Several mechanisms may contribute to type-specific vulnerability, including differences in the expression of calcium-permeable receptors (for example pannexin-1, P2X7, AMPA and transient receptor potential vanilloid receptors), the relative proximity of RGCs and their dendrites to blood supply in the inner plexiform layer, as well as differing metabolic requirements of RGC types. Such differences may make certain RGCs more sensitive to intraocular pressure elevation and its associated biomechanical and vascular stress. A greater understanding of selective RGC vulnerability and its underlying causes will likely reveal a rich area of investigation for potential treatment targets.

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[Introduction]

Glaucoma is a common cause of vision impairment and blindness, with an estimated 3.2 million people worldwide projected to be affected by 2020.[1] Major risk factors include advanced age, ethnicity and family history. It is commonly accompanied by elevated intraocular pressure (IOP), though this may not always be the case. If detected early, intervention can slow vision loss. Current treatments that lower IOP are generally successful, however, a significant proportion (~45 per cent) of people still show progressive visual field loss.[2] This indicates that factors contributing to disease onset and progression remain poorly understood.

Damage to the retinal ganglion cells (RGCs) is responsible for the vision loss in glaucoma. RGCs are the output neurons of the retina which send visual information along their axons to the brain. These projection neurons can be divided into a multitude of types, which process different features of the visual world.[3]

An emerging finding in glaucoma studies is the vulnerability of particular RGC types to damage. Studies from several independent groups support this idea, however, the mechanisms underlying selective RGC vulnerability are largely unexplored. The aim of this review is to examine the evidence for RGC type-specific vulnerability in human and experimental glaucoma. The potential mechanisms of selective vulnerability will also be described.

Glaucoma

Glaucoma describes a group of progressive neuropathies characterised by the degeneration of RGCs and their axons, which clinically results in ‘cupping’ of the optic disc and irreversible vision loss.[4] Open-angle glaucoma is the predominant form of glaucoma, accounting for approximately 75 per cent of cases.[5] It is characterised by an unobstructed irido-corneal drainage angle and a gradual remodelling of the connective tissue support at the optic nerve head.[6]

Glaucoma can be associated with, but not defined by age-related IOP elevation, caused by increased resistance to aqueous outflow through the trabecular meshwork. [4]

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In the absence of IOP elevation it is classified as normal-tension glaucoma. The following review will focus primarily on human studies pertaining to open-angle glaucoma and animal studies documenting RGC damage stemming from elevated IOP.

The pathology of open-angle glaucoma can be understood in terms of the biomechanics of the optic nerve head and RGC morphology. RGC compartments are spread throughout the inner retina; their dendrites, cell somata and axons residing in the inner plexiform layer, ganglion cell layer and nerve fibre layer, respectively (Figure 1A). These layers are highly vascularised by the intermediate and superficial vascular plexi (Figure 1B). Axons of RGCs exit the eye through the lamina cribrosa, a sieve-like network of connective tissue at the optic nerve head, then bundle together to form the optic nerve.[7]

With IOP elevations, the RGCs and tissues surrounding the lamina cribrosa suffer the highest levels of strain and posterior deformation, resulting in a gradual remodelling of both lamina cribrosa and peripapillary connective tissues and their supporting blood vessels.[8] This biomechanical strain and reduced blood perfusion can directly damage RGC axons. In addition, IOP-related strain can also modify RGCs at their dendrites as channels sensitive to membrane stretch are found throughout the cell. There is evidence that different types of RGCs are affected by mechanical stress directly or indirectly, and the different underlying mechanisms investigated in the field will be presented below.

Retinal ganglion cells type-specific damage in human glaucoma

The first evidence of RGC type-specific vulnerability in glaucoma arose from histological studies of human eyes. In a small cohort of 12 patients with open-angle glaucoma, large optic nerve fibres were disproportionately lost compared to smaller fibres, indicating that larger RGCs were more vulnerable to increased IOP.[9] Kerrigan-Baumrind et al.[10] found that glaucomatous eyes displayed a greater loss of larger diameter axons compared to smaller diameter axons. A follow up study noted a tendency for larger RGCs to be lost in eyes with glaucoma.[11] A criticism of these studies is that RGC soma shrinkage during glaucoma may lead to an underestimation

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of large RGCs and skew counts towards smaller RGCs.[12] These studies were also unable to make a clear distinction between RGCs and displaced amacrine cells (which are small in diameter), as they both reside in the ganglion cell layer of the retina.

Visual pathways that connect between RGCs and the lateral geniculate nucleus have been described in humans and primates as the koniocellular, the parvocellular and the magnocellular pathways. These pathways process different visual information; for example, parasol RGCs are large cells that contribute to the magnocellular pathway, and these cells are sensitive to moving stimuli and lower contrasts. Whereas midget RGCs are smaller cells that contribute to the parvocellular pathway, and these cells are more sensitive to colour and fine details.

Early psychophysical studies appeared to support histological findings that larger RGCs (for example, parasol cells) were more vulnerable in glaucoma by showing that the magnocellular pathway appeared disproportionately impaired. For example, Anderson et al.[13] found that visual acuity was significantly worse in patients responding to high temporal frequency light stimuli, a condition under which the magnocellular pathway predominates, compared to stationary stimuli targeting the parvocellular pathway. Sun et al.[14] also found reduced contrast sensitivity in the magnocellular pathway of glaucoma patients while responses favouring the smaller RGCs parvocellular pathway response were unaffected.

Another technique for assessing RGC function involves recording visually evoked potentials from the occipital cortex to stimuli that favours either magnocellular or parvocellular pathways. Howe et al.[15] found, using stimuli that target the magnocellular pathway in patients with open angle glaucoma or ocular hypertension, there was a reduction in the visually evoked potential response amplitudes and their contrast sensitivity. Furthermore, by separating the magnocellular and parvocellular components of the visually evoked potential, Klistorner et al.[16] identified a reduction in the magnocellular component in those with early glaucoma, while responses in the parvocellular component declined in those with more severe glaucoma.

More recent research using psychophysical testing, however, has not demonstrated clear differences between magnocellular and parvocellular pathway

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function, with similar levels of reduction in the sensitivity of both pathways reported among glaucoma patients.[17-19] Concurrent testing of magnocellular RGCs (using frequency doubling perimetry, motion-automated perimetry and flicker testing) and the RGCs responsible for short wavelength cone vision have indicated visual field defects compared to controls, but no preferential loss of one pathway over another amongst glaucoma patients.[20-23] Furthermore, patients in the early stages of glaucoma did not appear to differ to age-matched controls when presented with psychophysical stimuli that probe spatial summation of magnocellular or parvocellular pathways.[24] Whilst these studies find that magnocellular and parvocellular pathways show similar functional deficits early in glaucoma, compensatory mechanisms may mask selective injury to particular RGC types.

Criticisms of functional and histological studies point to the tendency for "selective" stimuli or imaging analysis to bias detection of changes towards a particular RGC type.[12,25] Efforts to functionally separate magnocellular and parvocellular pathways are complicated by an absence of stimuli which stimulate both pathways in comparable ways. Thus, the question of whether certain RGC types are more vulnerable in human patients with glaucoma remains contentious.

Retinal ganglion cell types

Understanding the mechanisms for how RGCs lose function and die during glaucoma can be approached by first examining RGCs fundamentally. Though animal models have been invaluable in refining our knowledge of RGC types, cell classification across species is highly heterogenous and is further complicated by different naming conventions used between species.

The earliest study of mammalian RGCs noted that they possess a circular receptive field that responds to increments and decrements of light, termed ON and OFF cells, respectively.[26] Knowledge of RGC function has now expanded to include up to 32 types based on their responses to a host of complex visual stimuli such as: directional selectivity, chromatic tuning, edge detection, and intrinsic photosensitivity.[3,27] For most basic investigations of RGC function, mouse RGCs are

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divided into ON, OFF or ON-OFF, and sustained or transient types which adequately describe the majority of RGCs recorded in most experimental settings.[28-30]

Anatomically, RGC dendritic arbours stratify in the inner plexiform layer at different layers depending on their response to light. An early examination of stratification patterns revealed OFF-RGC dendritic arbours terminate in the outer two-fifths of the inner plexiform layer, synapsing with OFF-bipolar cells; whilst ON-RGC dendrites terminate in the inner three-fifths, synapsing with ON-bipolar cells.[31] Recently, more precise analysis identified at least 37 RGC types as each type displayed stratification in distinct layers of the inner plexiform layer, correlating with their function.[32]

Identifying genetic markers for RGC types can aid in understanding their function and morphology, especially when combined with transgenic mice. One of the best-studied types in mice is the αRGCs, likely due to their large size. This group of large-bodied RGCs with large dendritic fields and rapid conduction are known as Y-cells in cats and correlate to parasol cells in primates.[3] They can be identified with KNCG, SMI-32 and osteopontin in mouse retina.[28,33]

Another well-established marker is melanopsin, which identifies the intrinsically photosensitive RGCs.[34] Though the highly complex work of characterising RGC types through genetic markers is still in its infancy, the advent of single-cell RNA sequencing, RGC type-specific markers may be more readily explored.[35] Recently, a study using molecular techniques to classify retinal cell classes in primate retina found 16-18 RGC types.[36] Interestingly, several glaucoma-associated genes were enriched in differing RGC types. For example, *MEIS2* showed higher expression on OFF compared with ON RGCs, and *SIX6* showed greater expression in magnocellular compared with parvocellular RGCs. Thus elucidating molecular signatures for RGC types would have wide-ranging implications for experimental and clinical research.

Retinal ganglion cells type-specific damage in animal models

Non-human primate models

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To better understand the effects of IOP elevation in a comparable species, several studies have modelled glaucoma by laser treating the trabecular meshwork of non-human primates. This treatment impedes the aqueous outflow, leading to increased IOP. These primates possess a lamina cribrosa like humans and can model the effects of elevated IOP on the biomechanics of the optic nerve head. Like in human glaucoma, cynomolgus monkeys with laser-induced IOP elevation were shown to have fewer RGCs with large somata and fewer large diameter axons in the optic nerve.[37] An immunohistochemical study showed RGCs had reduced neurofilament staining, which is indicative of large RGC types.[38] Furthermore, parasol RGCs have exhibited subtle shrinkage of their somata, axons and dendritic field before observing changes in midget RGCs.[39]

The visual pathways between RGCs and the lateral geniculate nucleus of non-human primates consists of layers that correspond to either the magnocellular or parvocellular pathway, much like the human lateral geniculate nucleus. Multiple studies using models of glaucoma in non-human primates have shown that dysfunction of afferent parasol or midget cells leads to degeneration of the magnocellular or parvocellular pathway, respectively, as reviewed by Yücel.[40] Following two weeks of increased IOP, the magnocellular layers of the lateral geniculate nucleus with input from the glaucomatous eye appeared to contain fewer and smaller cells compared to the parvocellular layers.[41] However, equal reductions in magnocellular and parvocellular pathways were observed when examining metabolism, neurofilament and synapses in the lateral geniculate nucleus after almost one year of elevated IOP.[42,43]

As with human findings, primate studies are inconclusive regarding the vulnerability of the magnocellular pathway. This variability may be due to the duration of IOP injury. Chronic IOP elevation would likely lead to an equal degeneration of the magnocellular and parvocellular pathways which is seen late in the disease, whilst magnocellular pathways alone may be more vulnerable following acute IOP injury which may mimic the early disease state.[9-11,41]

Cat models

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Cats have Y- and X-RGCs which are analogous to primate parasol and midget RGCs, respectively.[3] The Y-cells have larger somata, dendritic fields and thicker axons and dendrites, and respond best to low contrast and low spatial frequency. By contrast, the X-cells have small, highly-branched dendritic fields, medium somata and respond best to high contrast and high spatial frequency.

A series of cat retina studies have consistently noted type-specific vulnerability to elevated IOP. Following chronic IOP elevation (up to 1 month), investigators noted that the larger RGC cells (the Y-cells) had a lower density, shrinkage of dendritic fields and fewer bifurcations in remaining Y-cells.[44] Interestingly, the same group studied acute elevation of IOP (lasting from 1 min to 20 min, up to 90 mmHg with a hydraulic system or through cannulation) and observed that Y-cells maintained their firing rate in response to increasing pressure, whereas X cells showed a reduction in firing rate even at lower pressures.[45,46] Though this would seem paradoxical in the context of chronic IOP elevation, it may be that, large and small RGCs have differing susceptibilities and adaptation mechanisms in response to acute or chronic injury.

Mouse models

Mouse models have been valuable tools for modelling the effects of elevated IOP on RGCs as they can be used acutely, chronically and in conjunction with specific transgenes that label individual RGC cell types.[3] In addition, a range of methods to experimentally induce elevated IOP by impeding aqueous humour drainage allows modelling of glaucomatous RGC type-damage in mice.[47] Inducible approaches include episcleral vein occlusion/cauterisation, injection of microbeads into the anterior chamber, laser-induced damage of the trabecular meshwork and circumlimbal suture compression of episcleral veins.[47,48] A commonly used genetic model is the DBA/2J mouse which mimics pigmentary glaucoma and exhibits increased IOP at ~6-9 months of age.[49]

A common finding among mouse studies has been the vulnerability of OFF RGCs during experimentally-induced IOP, as reviewed by Della Santina and Ou.[50] Similar to findings in non-human primates and cats, dendritic field shrinkage was one of

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the earliest observations in mice and occurred in OFF-transient RGCs prior to other RGC types.[29,51,52] OFF cell types also had reduced receptive field size prior to other RGC types following increased IOP, likely due to dendritic field shrinkage.[29,52] Furthermore, in eyes with increased IOP, OFF cells exhibited a lower firing rate compared to control eyes after a full field stimulus.[53] Conversely, some studies have found no bias in dendritic shrinkage of RGC type, as both ON and OFF RGCs have shown dendritic pruning following two weeks[54] and over a month of IOP elevation[55]. Additionally, a study in cat retina noted a greater reduction of responses in ON cells compared to OFF cells after an acute increase in IOP.[46]

The variance in findings highlight the potential for model- and species-related differences. Whilst most models discussed here have maintained an average IOP increase of ~10 to 15 mmHg[29,51-53], different methods of increasing IOP range from mild (~5 mmHg) to severe (~90 mmHg). Another factor to consider is the timepoint at which RGCs are assessed, which can range from seven days[51] to 1.5 months[56] after IOP elevation, and may present differing snapshots of the degeneration process.[50] When comparing across species, different RGC types, optic nerve architecture and vasculature are important considerations as they may affect our understanding of glaucoma in humans. Notably, mice do not possess a lamina cribrosa consisting of connective tissue, instead they have a similar structure consisting of astrocytes ensheathing the axons of the optic nerve.[57]

Other models inducing RGC stress support the observation that OFF RGCs are particularly vulnerable. Optic nerve crush (ONC, which induces a much more severe RGC axonopathy)[47] has a greater impact on attenuating peak firing rate and receptive field size in OFF cells compared with ON cells.[30] Transgenic mice, with fluorescently-labelled RGC types, subjected to ONC also showed OFF-transient αRGCs were most vulnerable to injury, whilst melanopsin-containing RGCs were the most resistant.[58]

Potential mechanisms underlying RGC-type vulnerability

Whilst differences between species exist and certain studies fail to observe selective dysfunction or loss, there is compelling support for type-specific vulnerability of RGCs.

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Differences between RGCs in receptor expression, metabolic usage or external vascular and biomechanical environment, may help to explain RGC type susceptibility to IOP injury (Figure 2).

Differential receptor expression

Expression of patterns of receptor types underlie difference in neuronal function. Presence of receptors which encourage excitotoxicity (through allowing calcium entry) may make certain RGCs more vulnerable to damage. This section will explore current evidence for RGC type-specific expression of receptors that may lead to increased susceptibility to IOP related injury.

THE PANNEXIN-1 AND P2X7 RECEPTOR COMPLEX

Pannexin-1 channels are low resistance membrane proteins expressed throughout vertebrate tissues, including the rodent retina.[59] Pannexin-1 channels consist of six transmembrane subunits which allow ATP to exit and cations, such as calcium, to enter following stimulation by membrane stretch or P2X7 receptor agonism.[59]

Pannexin-1 activation on RGCs may contribute directly to RGC cell death. Within mouse retinae, pannexin-1 has been located on RGC somata and processes.[60] Pannexin-1 knockout mice displayed reduced neuronal cell death *in vitro* and RGC death *in vivo* following ischemic injury.[60] Furthermore, recent findings indicated heterogenous expression of pannexin-1 channels on RGCs, and greater pannexin-1 function was apparent in OFF RGCs.[61] While this could lead to greater calcium influx in OFF RGCs, causing their increased vulnerability following injury, there is evidence that pannexin-1 does not act alone. Studies of cultured cells found both pannexin-1 and the P2X7 receptor were necessary for inducing a large inward current[62,63] which could be capable of potentiating RGC death through excitotoxicity.

The P2X7 receptor is a trimeric ion channel that, upon stimulation by ATP, allows sodium, potassium and calcium to flow across the membrane.[64] They have been found on RGCs, as well as amacrine, horizontal and photoreceptor cells.[65,66] Compared to other purinergic receptors, P2X7 is unusual as it requires a higher concentration of ATP to activate, shows minimal desensitization and larger molecules can enter the cell following extended agonist application, likely through the pannexin-1 channel.[62-64]

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These characteristics of the P2X7 receptor suggest it may play a role under pathological conditions where the extracellular milieu contains high concentrations of ATP and calcium from dying cells.

There has been evidence that the P2X7 receptor exerts an asymmetrical effect in ON and OFF pathways under physiological conditions. Kupenova et al.[67] found the P2X7 receptor contributed more to the OFF-pathway response than the ON-pathway. Taken together, these studies may point towards a greater vulnerability in the OFF pathway under pathological conditions due to greater P2X7 receptor and pannexin-1 expression or function. While the P2X7 receptor has been implicated in potentiating RGC death following increased IOP[68,69] and optic nerve crush,[70] the importance of the P2X7 receptor in different RGC types under pathological conditions remains unassessed.

CALCIUM-PERMEABLE AMPA-RECEPTORS

Glutamate is a vital excitatory neurotransmitter for RGCs as they receive input from bipolar cells. RGCs express a host of ionotropic and metabotropic glutamate receptors on their dendrites – the *N*-methyl-D-aspartate and AMPA receptors being particularly relevant. Excitotoxic injury usually involves the *N*-methyl-D-aspartate receptor as it mediates calcium entry,[71] however, there has also been evidence that calcium-permeable AMPA receptors can be expressed under stressful conditions and lead to large calcium influxes that trigger RGC remodelling and cell death.[72,73]

A recent approach to understanding excitotoxic mechanisms in glaucoma involves examining local changes in glutamate arising from its insufficient extracellular clearance.[72] As human and animal studies have shown a downregulation of glutamate transporters,[74-76] this may underlie excitotoxic RGC death, which has been shown to be caused by increased extracellular glutamate.[71,77]

Difference in AMPA receptor subunit expression on RGCs may contribute to RGC type vulnerability to stress. Following excitotoxic injury, AMPA receptors on RGCs appeared to become calcium-permeable.[78] Recently, Wen et al.[79] examined AMPA receptor composition on different RGC types using dissociated RGCs. They found that compared to ON RGCs, OFF and ON-OFF RGCs showed higher calcium-permeable

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AMPA receptor currents and show greater calcium influx after pressure-induced stress. In contrast, Jones et al.[80] report that ON RGCs show higher expression of calcium-permeable AMPA receptors under baseline conditions. Thus, calcium-permeable AMPA-mediated RGC type vulnerability to IOP elevation requires more research to understand its relevance and role in RGC death.

TRANSIENT RECEPTOR POTENTIAL VANILLOID CHANNELS

Transient receptor potential vanilloid channels are cation-selective channels that sense a variety of stimuli in their cellular environment including, critically for glaucoma, mechanical stretching of cell membranes.[81] Recent studies have shown that transient receptor potential vanilloid channels may play a role in glaucoma as transient receptor potential vanilloid-1 and -4 channels are highly expressed in the ganglion cell layer.[82-84] Ryskamp et al.[84] showed that transient receptor potential vanilloid-4 channels were densely localised at the optic nerve head, the purported initial site of damage in glaucoma. Jo et al.[85] showed that there is a non-uniform distribution of transient receptor potential vanilloid-1 channels in the retina, suggesting the potential for preferential expression of this receptor in some RGC types. Furthermore, Lakk et al.[86] showed that a subset of RGCs, the αRGCs, co-expressed transient receptor potential vanilloid-1 and -4. This combined expression may contribute to greater vulnerability of these large RGCs to IOP-related injury.

Transient receptor potential vanilloid-1 and -4 channels may contribute to RGC death by increasing intracellular calcium.[83,84] Ryskamp et al.[84] found RGCs exhibited increased intracellular calcium following a hypotonic stimulation that caused their membranes to stretch.[84] In this *in vitro* model of mechanical stress, calcium influx was mediated by transient receptor potential vanilloid-4 channels. Transient receptor potential vanilloid-1 channels may also be involved in sensing IOP as Sappington et al.[83] showed in DBA/2J mice increased expression when IOP became mildly elevated.

In cultured RGCs, the build-up of intracellular calcium and apoptosis following elevated pressure was attenuated when transient receptor potential vanilloid-1 channels were blocked. However, the role of transient receptor potential vanilloid-1 in

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RGC death is not clear as Ward et al.[87] showed that mice lacking transient receptor potential vanilloid-1 channels exhibited accelerated RGC loss following exposure to chronic IOP elevation. The roles of transient receptor potential vanilloid-1 and -4 in mediating RGC type vulnerability continues to be an area of research interest.

Implications of vascular perfusion in retinal ganglion cells dysfunction

In addition to endogenous RGC type-specific factors, the role of the local vasculature may also play a role in RGC sensitivity. The mouse retinal vasculature mainly consists of 3 parallel but interconnected plexi (see Figure 1B). The superficial vessel complex supplies the nerve fibre layer and the ganglion cell layer, whilst the intermediate capillary plexus is located at the junction between the inner nuclear layer and inner plexiform layer which coincides with the dendrites of the OFF RGCs (Figure 1B). The deep capillary plexus is at the junction of the inner nuclear layer and the outer plexiform layer (Figure 1B). Retinal neurovascular coupling is a well-documented phenomenon whereby increased neuronal activity results in capillary dilation.[88] Given the high energy demand of neurotransmission through the inner retina, the proximity to the vasculature is an important consideration.

Human and animal studies of glaucoma consistently report that poor perfusion may contribute to RGC dysfunction and death in glaucoma. In glaucoma patients, a 10-year longitudinal study showed narrowing of blood vessels appeared to precede the onset of glaucoma.[89] Several epidemiological studies have highlighted the potential importance of reduced ocular perfusion pressure to contribute to glaucoma progression.[90,91] Ocular perfusion pressure describes the pressure required to drive blood through intraocular vasculature. A lower perfusion pressure throughout the day, combined with high IOP, was associated with reduced retinal vessel density[92] and may mean reduced intraocular blood flow. Optical coherence tomography angiography has demonstrated all retinal capillary layers in glaucoma patients demonstrated reduced vessel density.[93]

Animal models demonstrate similar vasculature abnormalities, however, it is unclear whether these changes are a cause or a consequence of RGC functional loss.

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Capillary volume is decreased in primates following months of moderate IOP.[94] Experimental glaucoma in rats showed reduced capillary volume, perimeter, diameter and density in regions of the optic nerve.[95] Similarly, DBA/2J mice have reduced choroidal and retinal blood flow associated with increased IOP at nine months of age.[96] Given that OFF RGC dendrites are close to the intermediate capillary plexus one might expect that they are better perfused. In addition, in mice, a sparse fourth intersublaminal vascular plexus has been observed in the inner plexiform layer, at the interfacing layer between OFF transient and OFF sustained terminals (Figure 1B).[97] Although the intermediate capillary plexus is less dense than the deep capillary plexus, it appears to show greater vasodilation in response to flickering light in the rat retina.[98] This larger response may reflect a higher metabolic demand in the OFF pathway with neurotransmission.

The M1 melanopsin RGC type may be important in comparing the role of vasculature and function as they stratify in the OFF sublamina whilst receiving ON-bipolar cell input.[99] As M1 RGCs have shown reduced dendritic branching a week after IOP injury, this suggests dendrites in the OFF sublamina are vulnerable.[52] Thus changes to blood vessels, reductions in blood flow and impaired perfusion might have a greater impact on the OFF sublamina compared with the ON sublamina.

Metabolic differences in RGC types

RGC axons, particularly the unmyelinated sections, exhibit an increased density of mitochondria when compared to other retinal cell types[100] likely reflecting the high energy needs of conducting action potentials in RGCs.

Increased IOP is known to disrupt mitochondrial transport and alter cytochrome C oxidase expression in RGCs, reflecting altered metabolism. Takihara et al.[101] showed that RGC axons, imaged in vivo, had reduced mitochondria number, length and mobility following increased IOP. Importantly, this reduction occurred prior to RGC loss. Furthermore, accumulation of mitochondrial DNA mutations and loss of respiratory enzyme activity was observed following increased IOP, and this was accompanied by RGC loss.[102] Finally, as modification of mitochondrial function reduces RGC

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vulnerability to IOP elevation in older mice,[103] these data suggest that metabolism and energy production in RGCs is an early indicator of dysfunction following IOP elevation.

Studies of central and enteric nervous systems show that mitochondrial abnormalities lead to loss of specific types of neurons suggesting that differences in energy use can impact neuronal vulnerability.[104-106] Metabolic differences between ON and OFF RGCs are apparent in their baseline functional differences. Experimentally, prior to light stimulation, OFF cells displayed higher spiking activity, more frequent increases in intracellular calcium and larger excitatory post-synaptic potentials compared to ON cells.[107,108] OFF cells also have a lower spike threshold.[107] Additionally, the difference in metabolic activity of OFF and ON RGCs has been probed with cytochrome C oxidase staining. In cat, ferret and macaque retinae, large-bodied RGCs and the OFF sublamina of the inner plexiform layer were observed to be darkly stained, indicating highly metabolically active neurons in these regions.[109] Taken together the above findings suggest the OFF-RGCs are more active, requiring greater energy demands, and may be at greater risk during stress. Highlighting the species differences, primates showed an opposing staining pattern as darker staining was apparent in the ON-sublamina of the inner plexiform layer, suggesting a metabolically more active ON pathway. The authors highlight the complexities of this interpretation, as primate ON and OFF RGCs are not as distinctly separated when compared to ferrets, and primates have a greater reliance on colour vision thus potentially using differing retinal circuitry.

Investigations of the authors in normal mouse and rat retina do not indicate a clear difference in labelling of cytochrome oxidase C between the ON and OFF-sublaminae in these animals (Figure 3). In accordance with Kageyama et al.,[109] large cells appear to be darkly stained possibly due to greater metabolic activity (Figure 3E-H); however, a more detailed morphological analysis is required to confirm this possibility. Further investigation to elucidate the metabolic demands of RGC types could be conducted through combining cytochrome C labelling with type-specific staining or using transgenic mice.

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In understanding intrinsic mechanisms of RGC vulnerability it is also helpful to examine mechanisms that make RGCs more resilient to damage. In metabolic diseases of patients and mouse models of Leber's hereditary optic neuropathy and dominant optic atrophy, intrinsically light-sensitive melanopsin RGCs were preserved.[99,110] Melanopsin RGCs are large RGCs that express the light-sensitive protein melanopsin, and project to the hypothalamic suprachiasmatic nucleus which is involved in control of circadian rhythms. In optic nerve transection or crush injury, melanopsin RGCs were more resistant than other RGCs to cell death and constituted the majority of the remaining RGC population.[33,58,111,112] Melanopsin RGCs were also resistant to cell death during IOP injury[113-115], despite having shown reduced dendritic branching.[52]

Why this subclass might be less vulnerable is not understood. Melanopsin RGCs stain more intensely for cytochrome C oxidase compared to other RGC types suggesting they contain significantly more mitochondria.[116] It has been suggested that a functional mitochondrial deficit may be compensated for by high mitochondria numbers; however, it is unclear whether mitochondrial density is proportional to the large size of melanopsin RGCs. It is likely, due to their size, melanopsin RGCs require high numbers of mitochondria to support the information transfer from the large dendritic arbours to the terminals,[117] which would suggest they are, in fact, more vulnerable to mitochondrial deficits. Furthermore, RGCs with more mitochondria would have a greater reliance on mitophagy (a mechanism to clear spent mitochondria),[118] transcellular mitophagy[119] and mitochondrial biogenesis,[120] all of which could lead to increased energy demands.

The observation that melanopsin RGCs are less vulnerable to IOP elevation is in contrast to the earlier findings that there was greater loss of larger RGCs and axons in humans and primates,[9,10,11] but congruent with studies in rodents that identified mRGCs and the large αON sustained RGCs being more resistant to injury.[58,112] Indeed, αON RGCs have been shown to express melanopsin, albeit at very low levels and may be considered a melanopsin RGC type.[121] Thus, more work is required to understand why melanopsin RGCs are more robust compared to other RGC subclasses.

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Ageing effects on metabolism

Mitochondrial DNA deletions accumulate with advancing age in rodent and human retinae. [122,123] Mitochondrial DNA in aged mice and rats showed increased oxidative damage compared to young animals, corresponding to reduced transcript and protein expression of DNA repair enzymes.[123] Accumulation of mitochondrial DNA damage was localised to photoreceptors and the ganglion cell layer.[120,123]

Decreased mitochondrial function has been observed in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntingtin's disease.[124] In these diseases, age-related neurodegenerative loss is brain region and neuronal type specific. With age, the mouse optic nerve has been shown to demonstrate a slowed transport of metabolic cargoes and mitochondria.[125,126] In addition, mitochondrial transport was significantly slowed in the optic nerve of a mouse model of Parkinson's disease;[126] however, differences of metabolic transport between RGC types was not examined. Alternatively, the reduction in transport is due to a reduction in dendritic arbour size. Samuel et al.[127] found that mouse RGCs showed a ~20 per cent reduction of soma size and ~13 per cent reduction in dendritic field during ageing. Although, whether OFF cells age differently to ON cells is not known, there is evidence that different neuronal classes do age differently.[127]

Age and IOP elevation concurrently alter RGC metabolism. Older mice (14–17-month-old) with fluorescently-tagged mitochondria had lower mitochondrial density compared to young (four-month-old) mice.[128] Ju et al. [129] found that IOP elevation further decreased mitochondria density in DBA/2J young and old mice compared to wildtype mice. The concentration of ATP, the energy source produced by mitochondrial respiration, was also reduced in older DBA/2J mice.[130] Additionally, RGC recovery was shown to be hampered in older mice following a retinal injury. RGCs in old mice take longer to recover from acute IOP elevation and show reduced recovery following oxygen and glucose deprivation compared to young mice.[103,130] This suggests that ageing provides a metabolic insult that may heterogeneously affect RGC types, making some more susceptible to damage, which is then exacerbated by elevated IOP.

Conclusions

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Evidence of RGC type-specific vulnerability has steadily grown over several decades. While human findings remain controversial, most mouse studies show increased susceptibility of OFF RGCs (summarised in Figure 2) and preservation of melanopsin RGCs. RGC-intrinsic factors and extracellular factors may all play a role in determining type vulnerability. Further studies aimed at understanding the underlying mechanisms of RGC type-specific susceptibility to injury are required to better understand early stages of glaucoma and help to identify potential therapeutic targets.

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[Figure legends]

Figure 1. Retinal architecture and vascular plexi. A: The outer and inner segments (OS/IS) of photoreceptors (purple) and their nuclei in the outer nuclear layer (ONL) make up the outer retina. Nuclei of horizontal cells (green) and bipolar cells (orange) make up the inner nuclear layer (INL). Bipolar cell axons, amacrine cells (green) and RGC dendrites make up the inner plexiform layer (IPL). RGC (blue) nuclei reside in the ganglion cell layer (GCL) and their axons form the nerve fibre layer (NFL). Displaced amacrine cells are found in the GCL, making up ~50% of the cell population. The IPL is divided into sublamina a and sublamina b, corresponding to OFF and ON input from bipolar cells and, subsequently, dendrites of ON and OFF RGCs. The intermediate capillary plexus is located chiefly in sublamina a. B: Mouse transverse section stained with hematoxylin and eosin, showing the location of the vascular plexi. Optical coherence tomography angiography *in vivo* imaging of vascular layers in a mouse eye, *en face* (Spectralis OCT2, Heidelberge Engineering, Heidelberg, Germany). Images are collected across a 30 x 35 degree field, just superior to the optic nerve and segmented to show the deep capillary plexus (red box), intermediate capillary plexus (green box) and the superficial vessel complex (blue box). The approximate position of the intersublaminal plexus is marked by the white dashed line.

Figure 2. A: Under baseline conditions OFF RGCs show a higher firing rate compared to ON RGCs. OFF RGCs may also display greater P2X7 receptor (P2X7, green) and pannexin-1 (Panx1, blue) activity compared to ON RGCs. The greater metabolic activity of OFF RGC dendrites compared to ON RGCs is indicated by the dark blue shading. Transient receptor potential vanilloid receptors 1 and 4 (TRPV, purple) are present on large αRGCs which may be either ON or OFF. ON and OFF RGCs both express AMPA receptors (AMPA, orange) which are not normally permeable to calcium (Ca^{2+}, red circles). Blood vessels of the intermediate plexus are illustrated between the OFF sublamina of the IPL and the INL. B: Following IOP elevation, OFF RGCs usually show a reduced firing rate and dendritic field shrinkage, the pale shading indicating a potential loss of metabolic activity in OFF RGCs. ON RGCs may also have reduced firing rates. ATP released from damaged cells may stimulate the P2X7/pannexin-1

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complex and pressure can stimulate the transient receptor potential vanilloid receptors, leading to greater calcium influx. Additionally, AMPA receptors become calcium permeable (CP-AMPA) on OFF RGCs. The intermediate plexus shows reduced vessel density and blood flow and volume.

Figure 3. Cytochrome oxidase 1 (MTCO1, cat. no. ab14705; red) immunolabelling of A–D: mouse and E–H: rat retinae. A–D: a transverse section from a Thy1-HYFP mouse stained with A: MTCO1, B: GFP and C: DAPI. Note the intense MTCO1 staining (arrow) of the YFP^+ putative ON RGC compared to other cell bodies. E–H: dark agouti rat transverse sections with E, G: MTCO1 and F, H: DAPI. Intensely stained cells, most likely RGCs, appear to be larger than neighbouring cells. Scale bar: 20μm in A-F, B: 10 μm in G-H.

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CXO 19-130
INVITED REVIEW

Potential mechanisms of subtype-specific vulnerability of retinal ganglion cells in glaucoma

Wang

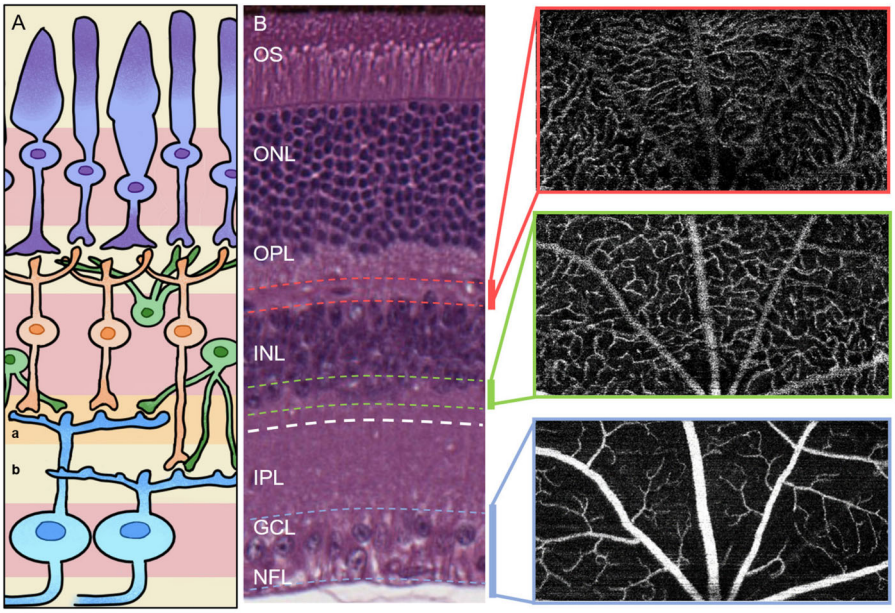


Figure 1. Retinal architecture and vascular plexi. A: The outer and inner segments (OS/IS) of photoreceptors (purple) and their nuclei in the outer nuclear layer (ONL) make up the outer retina. Nuclei of horizontal cells (green) and bipolar cells (orange) make up the inner nuclear layer (INL). Bipolar cell axons, amacrine cells (green) and RGC dendrites make up the inner plexiform layer (IPL). RGC (blue) nuclei reside in the ganglion cell layer (GCL) and their axons form the nerve fibre layer (NFL). Displaced amacrine cells are found in the GCL, making up ~50% of the cell population. The IPL is divided into sublamina a and sublamina b, corresponding to OFF and ON input from bipolar cells and, subsequently, dendrites of ON and OFF RGCs. The intermediate capillary plexus is located chiefly in sublamina a. B: Mouse transverse section stained with hematoxylin and eosin, showing the location of the vascular plexi. Optical coherence tomography angiography in vivo imaging of vascular layers in a mouse eye, en face (Spectralis OCT2, Heidelberge Engineering, Heidelberg, Germany). Images are collected across a 30 x 35 degree field, just superior to the optic nerve and segmented to show the deep capillary plexus (red box), intermediate capillary plexus (green box) and the superficial vessel complex (blue box). The approximate position of the intersublaminal plexus is marked by the white dashed line.

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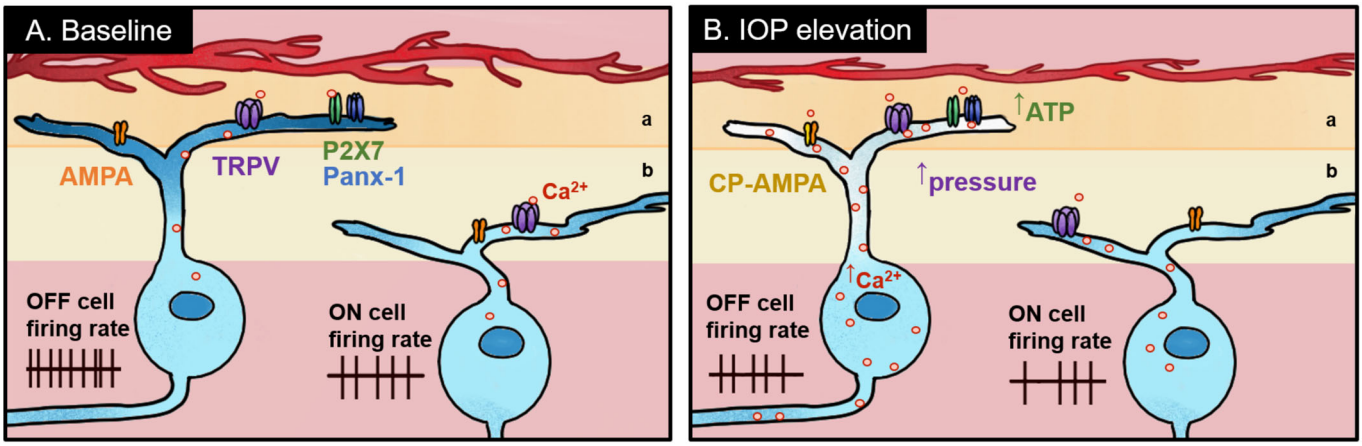


Figure 2. A: Under baseline conditions OFF RGCs show a higher firing rate compared to ON RGCs. OFF RGCs may also display greater P2X7 receptor (P2X7, green) and pannexin-1 (Panx1, blue) activity compared to ON RGCs. The greater metabolic activity of OFF RGC dendrites compared to ON RGCs is indicated by the dark blue shading. Transient receptor potential vanilloid receptors 1 and 4 (TRPV, purple) are present on large ŒRGCs which may be either ON or OFF. ON and OFF RGCs both express AMPA receptors (AMPA, orange) which are not normally permeable to calcium (Ca2+, red circles). Blood vessels of the intermediate plexus are illustrated between the OFF sublamina of the IPL and the INL. B: Following IOP elevation, OFF RGCs usually show a reduced firing rate and dendritic field shrinkage, the pale shading indicating a potential loss of metabolic activity in OFF RGCs. ON RGCs may also have reduced firing rates. ATP released from damaged cells may stimulate the P2X7/pannexin-1 complex and pressure can stimulate the transient receptor potential vanilloid receptors, leading to greater calcium influx. Additionally, AMPA receptors become calcium permeable (CP-AMPA) on OFF RGCs. The intermediate plexus shows reduced vessel density and blood flow and volume.

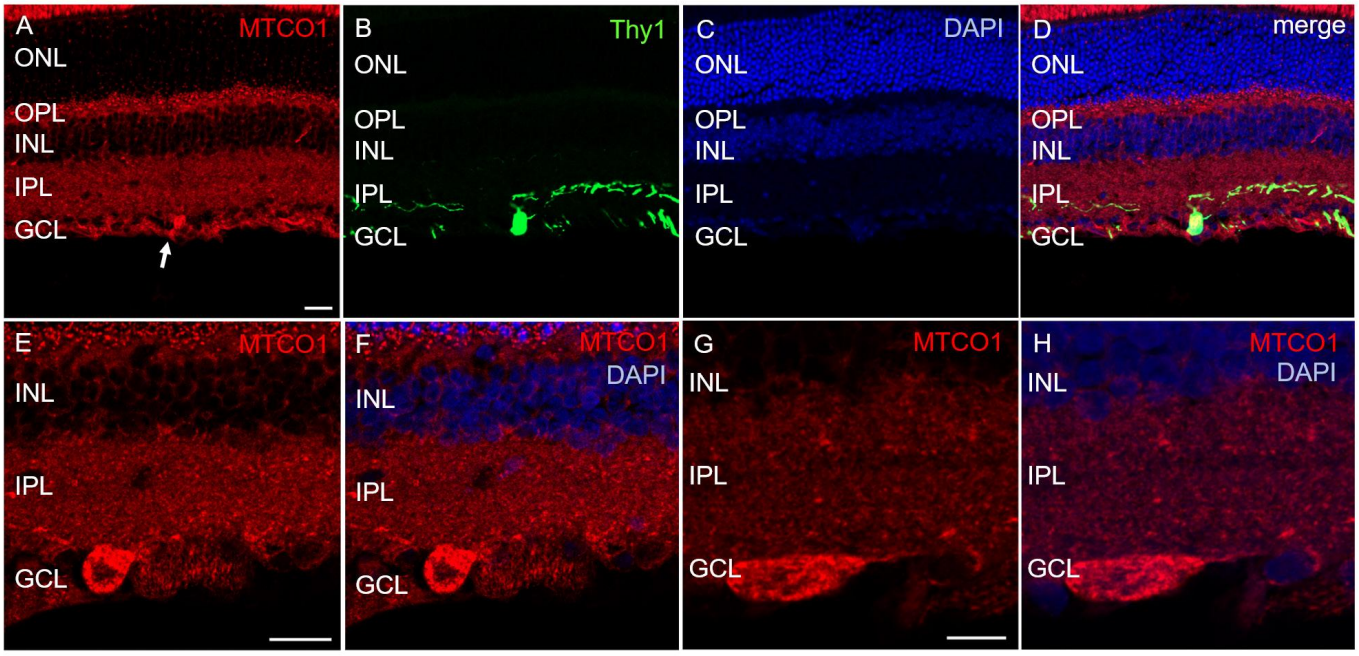
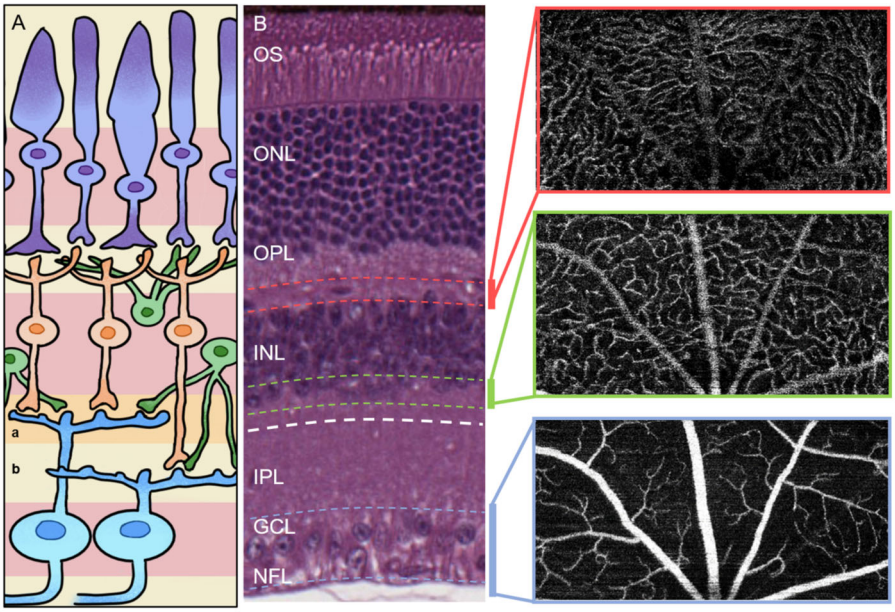


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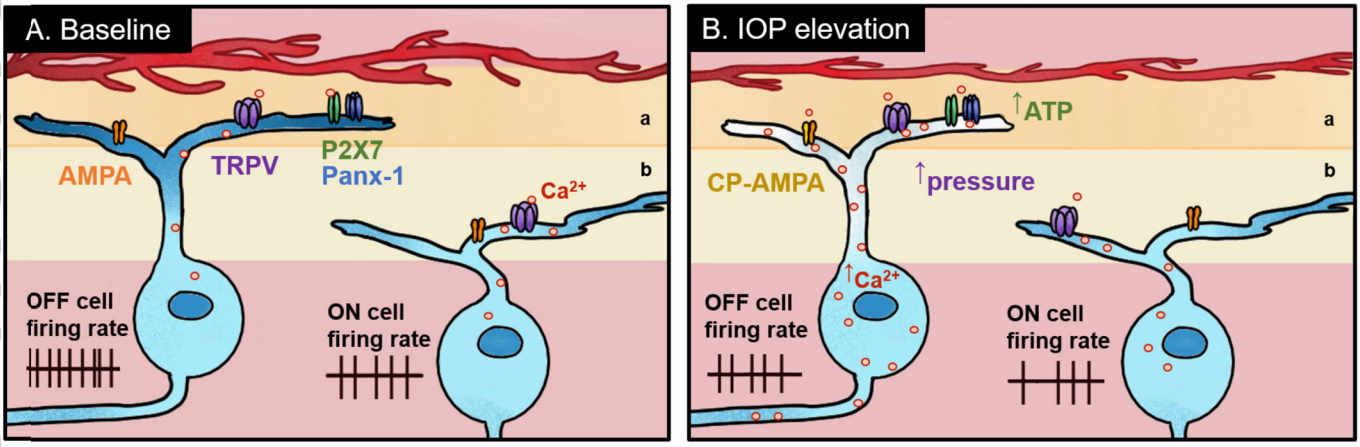
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CXO_13031_CXO 19-130 Figure 1 - F.tif

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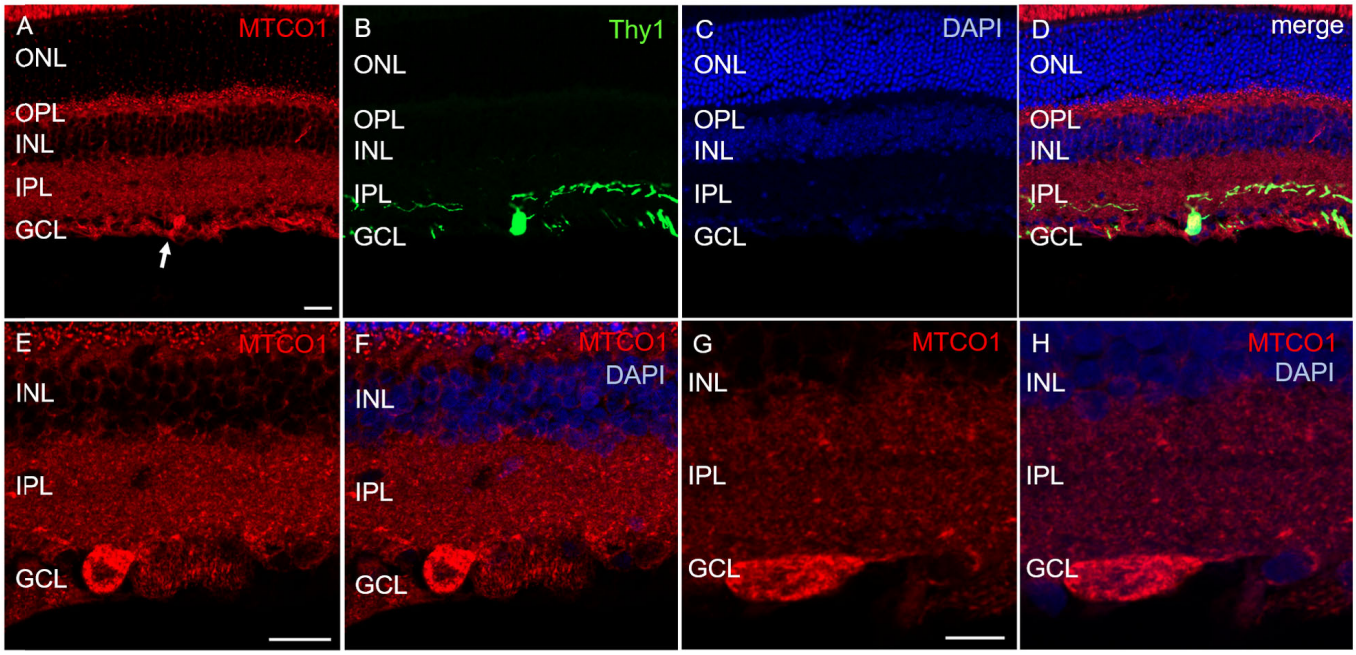
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CXO_13031_CXO 19-130 Figure 3 - F.tif

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