## Title
Who's lost first? Susceptibility of retinal ganglion cell types in experimental glaucoma



## Authors
Della Santina, Luca
Ou, Yvonne

## Publication Date
2017-05-01

## DOI
10.1016/j.exer.2016.06.006

Peer reviewed

HHS Public Access

Author manuscript

*Exp Eye Res*. Author manuscript; available in PMC 2018 May 01.

Published in final edited form as:

*Exp Eye Res*. 2017 May ; 158: 43–50. doi:10.1016/j.exer.2016.06.006.

# Who's lost first? Susceptibility of retinal ganglion cell types in experimental glaucoma

**Luca Della Santina**[a] and **Yvonne Ou**[b,*]

[a]Department of Pharmacy, University of Pisa, Via Bonanno, 6, Pisa, 56126, Italy

[b]Department of Ophthalmology, University of California, San Francisco, 10 Koret Way, San Francisco, CA, 94143, USA

## Abstract

The purpose of this article is to summarize our current knowledge about the susceptibility of specific retinal ganglion cell (RGC) types in experimental glaucoma, and to delineate the initial morphological and functional alterations that occur in response to intraocular pressure (IOP) elevation. There has been debate in the field as to whether RGCs with large somata and axons are more vulnerable, with definitive conclusions still in progress because of the wide diversity of RGC types. Indeed, it is now estimated that there are greater than 30 different RGC types, and while we do not yet understand the complete details, we discuss a growing body of work that supports the selective vulnerability hypothesis of specific RGC types in experimental glaucoma. Specifically, structural and functional degeneration of various RGC types have been examined across different rodent models of experimental glaucoma (acute vs. chronic) and different strains, and an emerging consensus is that OFF RGCs appear to be more vulnerable to IOP elevation compared to ON RGCs. Understanding the mechanisms by which this selective vulnerability manifests across different RGC types should lead to novel and improved strategies for neuroprotection and neuroregeneration in glaucoma.

### Keywords

Retinal ganglion cell types; Selective vulnerability; Glaucoma; Intraocular pressure; Dendrite; Synapse

## 1. The paradigm of selective vulnerability of RGC types in glaucoma

Dr. David Epstein framed the scientific questions in the field of glaucoma eloquently in the 4th edition of Chandler and Grant's Glaucoma (Epstein et al., 1997). He writes:

*Corresponding author. 10 Koret Way, Rm K323, UCSF Box 0730, San Francisco, CA, 94143, USA, yvonne.ou@ucsf.edu (Y. Ou).

**Conflict of interest**

The authors declare no competing financial interests.

**Animal rights**

All animal procedures were approved by the Institutional Animal Care and Use Committees at UCSF and the University of Washington, Seattle and were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals.

"In almost all cases an abnormality in drainage of aqueous humor through the outflow pathway tissue, potentially at many sites in the trabecular meshwork, that leads to elevation of IOP and causes damage to the optic nerve end organ, which demonstrates varying susceptibility to different levels of IOP (including statistically "normal" IOP). The two main scientific questions are: (1) in the open angle glaucomas what is the cause of obstruction to trabecular outflow and how can this **trabecular glaucoma** be best treated?; (2) what is the cause of the optic nerve damage and especially its varying **susceptibility** (a feature which can appropriately be termed an "optic neuropathy"), and are there any specific remedies for the optic nerve beyond consistently lowering the IOP? Both of these questions are very important. These two schools of scientific inquiry should be complementary rather than competitive."

While Dr. Epstein dedicated his research career towards elucidating our understanding of trabecular meshwork (TM) and aqueous outflow, as well as how to lower IOP by targeting the TM, he was also supportive of those of us who chose to study and potentially reverse the damage to the optic nerve. Almost three decades before the National Eye Institute announced its Audacious Goals Initiative to regenerate the axons of the optic nerve, Dr. Epstein wrote, "It is irrefutable that one would wish to prevent and reverse optic nerve damage with one's treatments, irrespective of curing the trabecular abnormality. This must be, in truth, a lofty and long-range goal that may require precedent knowledge from other areas of neurobiology and, in particular, those related to nerve regeneration." (Epstein, 1987). The varying susceptibility of the optic nerve is likely due to many different causes, and certainly much effort has been dedicated to uncovering the reasons for this phenomenon. A related concept in neurodegenerative diseases, selective vulnerability, is very much applicable to glaucoma. Selective vulnerability in the nervous system refers to the fact that subpopulations of neurons may be more or less vulnerable in response to injury (Saxena and Caroni, 2011). For example, in Parkinson's disease, the dopaminergic neurons of the substantia nigra selectively degenerate. In amyotropic lateral sclerosis, the upper and lower motor neurons are selectively vulnerable. For glaucoma, the retinal ganglion cells are the neuronal class most susceptible to various stressors such as elevated IOP. However, the potential mechanisms underlying selective vulnerability of neuronal sub-populations in neurodegenerative diseases are complex, multifactorial, and not yet completely understood. The same certainly holds true for glaucoma.

### 1.1. Are large RGCs more susceptible to injury?

While it is generally accepted that RGCs are the most vulnerable neuronal class in glaucoma, there has been controversy in the field as to whether certain RGC types are more or less vulnerable to IOP-induced injury. Earlier work in non-human primates and human tissue supported the concept that RGCs with the largest cell bodies and axons were the most susceptible to injury (Glovinsky et al., 1991; Quigley, 1999; Quigley et al., 1988, 1987). In the primate, two types of RGCs are the midget cells that comprise the P visual pathway ("P" cells) and the parasol cells that comprise the M visual pathway ("M" cells). Parasol cells have large receptive fields, high luminance and contrast sensitivity, and lack spectral sensitivity. Midget cells have smaller receptive fields, lower luminance sensitivity, and have spectral sensitivity. The first hint of selective loss among RGC types was from optic nerve

axon counts in the human and non-human primate. When compared to normal optic nerves, glaucomatous optic nerves had greater loss of large diameter axons (Kerrigan-Baumrind et al., 2000; Quigley et al., 1988, 1987). Subsequent work examining RGC size and rates of cell death in whole mount retina suggested that there was a greater reduction of larger diameter RGCs, which presumably give rise to larger diameter axons (Glovinsky et al., 1991). Quigley's group went on to show that axonal transport to the magnocellular layers was more impaired than to the parvocellular layers of the dorsal lateral geniculate nucleus in non-human primates with chronic IOP elevation (Dandona et al., 1991). Indeed, in a postmortem study of the lateral geniculate nucleus from glaucoma and control patients, the mean magnocellular cell density for the glaucoma group was significantly less than that for the control group, whereas there was no difference among groups in the parvocellular layer (Chaturvedi et al., 1993). Taken together, these experiments suggested that parasol cells of the M pathway are more susceptible to IOP-induced injury than midget cells of the P pathway, although one major caveat is that there was no definitive identification of parasol or midget cells as these studies relied on size classification alone. Indeed, one major critique is that the dendritic arbor and soma size of parasol and midget cells varies greatly depending on retinal eccentricity (reviewed in (Sample, 2001)). Therefore, broadly speaking, one could argue that RGCs with larger somata and larger axons were more vulnerable, but this did not necessarily indicate selective vulnerability of a specific RGC type.

The selective vulnerability of large axon or large somata RGCs came into question when other groups were unable to identify that these cells were the most susceptible in experimental glaucoma models. Using a retrograde labeling method to identify RGCs in a non-human primate model of ocular hypertension, Morgan et al. did not find a selective loss of parasol cells versus midget cells, although they did quantify a reduction in cell size for both types of surviving RGCs (Morgan, 1994). These experiments avoided the criticisms of the prior work, which included possible miscounting of RGCs due to displaced amacrine cells in the ganglion cell layer. This work also demonstrated that cell soma shrinkage was likely a stage of degeneration prior to cell loss, and called into question whether previous work misidentified large vs. small RGCs because of cell shrinkage. Of course, one major difficulty with all of these lines of investigation is that at the time, investigators were identifying RGCs solely by morphology, which alone is likely not enough to definitively specify RGC types. This will be addressed below (Section 3), as newer tools, especially in the mouse retina, have made the identification of RGC types more conclusive.

More recent investigations examining the function of these pathways are also conflicting with respect to the hypothesis that large field RGCs may be more vulnerable in glaucoma. Certainly, human autopsy studies and primate experimental glaucoma models suggested that neurons in the M layers of the LGN were more vulnerable than those in the P layers (Chaturvedi et al., 1993; Weber et al., 2000). Using fMRI, Zhang and colleagues found that early stage glaucoma patients were less responsive to transient achromatic stimuli than to sustained chromatic stimuli in the magnocellular layers of the LGN and the superficial layer of the superior colliculus (SC) but not in the P layers or cortical visual areas (Zhang et al., 2016). The authors conclude that early stage glaucoma causes selective functional loss of the larger cells in the human LGN and SC, specifically to stimuli modulated at high temporal frequencies. In contrast, psychophysical testing using a low-spatial-frequency contrast

sensitivity approach revealed that there was no selective loss of M or P function, nor greater loss of sensitivity of larger-field RGCs (McKendrick et al., 2007). One caveat in interpreting all of these studies, both experimental animal models and human studies, is that cross comparisons are challenging because of variations in the level of IOP elevation, stage of degeneration, specificity of measurement tool, and, in the case of experimental glaucoma, the strain or species and the model used.

### 1.2. Conflicts arise as to whether specific RGC types are selectively vulnerable

With the aid of RGC type specific labeling methods, several groups examined various types of RGCs in experimental glaucoma models. One of the first studies to utilize a relatively specific neuronal marker (SMI-32) that labels large somata RGCs rich in neurofilament found that these RGCs were more vulnerable compared to all RGCs in a non-human primate glaucoma model (Vickers et al., 1995). In rodent models of experimental glaucoma, there has also been conflicting data regarding selective vulnerability of specific RGC types to injury. Jakobs and associates examined several different neuronal types in the DBA/2J model of inherited glaucoma, in which IOP is elevated and RGCs degenerate in an asynchronous and chronic progressive manner (Jakobs et al., 2005). While the authors acknowledge the limitations of their study in terms of the small number of RGC types and the moderate to advanced stage of degeneration that were examined, they argue that there does not appear to be any vulnerability of a specific type nor any preferential loss of large RGCs. However, while the authors provided a qualitative description of various RGCs that were individually labeled, there was no quantification of specific RGC types except for RGCs labeled with SMI-32 (which brightly labels alpha RGCs or αRGCs with large somata and dendritic areas) and melanopsin-positive intrinsically photosensitive RGCs (also large soma and dendritic area RGCs). For both of these types the proportional loss was not different than other RGC types, but the retinas examined were graded moderate to severe degeneration by optic nerve axon counts. It is possible that at this late stage of disease there may not be an identifiable preferential loss as other cell types are also undergoing damage and apoptosis. Certainly, these experiments are unable to rule out the possibility that certain RGC types are vulnerable early in the course of degeneration while other types remain relatively resistant to damage until late in the disease.

Other studies have also examined specific types of RGCs to determine whether there is differential vulnerability to IOP elevation. Li and colleagues used a laser photocoagulation model in the rat to induce experimental glaucoma and investigated both the loss of melanopsin-expressing or intrinsically photosensitive RGCs (ipRGCs), as well as dendritic morphology (Li et al., 2006). Intrinsically photosensitive RGCs have large dendritic fields, and thus possibly could be predicted to be susceptible to injury. However, over all time points, which extended to 12 weeks, this RGC type appeared relatively resistant to injury, both in terms of total cell loss and dendritic complexity. Interestingly, the dendritic morphology of ipRGCs was quantified in the microbead occlusion model in mice, and the investigators found that while dendritic field area did not change 7 days after IOP elevation, there was a decrease in dendritic complexity (El-Danaf and Huberman, 2015). In summary, these investigations illustrate how challenging it is to address the question of selective vulnerability of specific RGC types in experimental glaucoma due to the inability to

specifically identify many different RGC types and examine them simultaneously. This is especially true given the fact that the delineation of RGC types in mammalian retina is still a work in progress.

## 2. The diversity of retinal ganglion cell types

The most recent estimate of the number of distinct RGC types found in the mammalian retina is around 30, with more than half of these types definitively identified (Sanes and Masland, 2015). However, the full catalog is not yet complete, and furthermore, even the tools used for classifying RGC types are either quite new or still under development. In their recent review, Sanes and Masland put forth four criteria for classifying RGCs: uniform morphology, similar gene expression, regular spacing, and uniform physiological properties. While this classification scheme appears relatively straightforward, the criteria of gene expression, as one example to demonstrate the challenge of classification, still has not been completely defined. For example, how many genes are required to specify a RGC type? Even so, by combining morphological, functional, molecular, and spacing criteria, there are likely at least 32 specific types in the mouse (Baden et al., 2016; Sanes and Masland, 2015; Sümbül et al., 2014).

Broadly speaking, RGCs can be functionally divided into ON-center versus OFF-center RGCs that respond to light intensity increases and decreases within the center of their receptive field, respectively. This functional distinction also correlates with anatomic stratification of the dendrites, whereby the ON and OFF RGCs arborize their dendrites in the inner and outer portions of the inner plexiform layer, respectively (Nelson et al., 1978). Indeed, one of the first types for which this structure function relationship was demonstrated were the alpha RGCs (αRGCs), first characterized by Wässle and colleagues (Cleland et al., 1975; Wässle et al., 1981). Physiologically, similar cells in other species include the Y cell in cats, the brisk transient cell in rabbits, and the parasol cell in nonhuman primates (reviewed in (Sanes and Masland, 2015)). In the mouse, there are three types of αRGCs (Pang et al., 2003), all with large somata, rich in neurofilament, and express *spp1* (encodes a secreted phosphoprotein osteopontin) and *kcng4* (encodes a voltage-gated potassium channel subunit) (Duan et al., 2015). These three types of αRGCs can be distinguished physiologically and morphologically based on the stratification level of the dendrites, with the ON sustained, OFF transient, and OFF sustained αRGCs stratifying around 30%, 50%, and 70% of IPL depth from the ganglion cell layer. Other well-characterized RGC types (reviewed in (Sanes and Masland, 2015)) include the directionally selective RGCs (ON, ON-OFF), intrinsically photosensitive RGCs, local edge detectors, and J-RGCs. Now that more powerful tools are available to define RGC types, we can leave the debate of whether large RGCs are more susceptible and examine each RGC type and define its specific susceptibility in a much more precise way.

## 3. Structural and functional perturbations of RGC types in experimental glaucoma

Although many advances have led to better definition and discrimination of the more than 30 RGC types in the mouse retina, it is still a challenge to define the rules that govern which

RGC types are most susceptible or resistant to glaucomatous injury in a comprehensive manner. However, several groups have attempted to address this challenge by utilizing various tools, including genetic, functional, and morphological criteria that permit more definitive type definition than in the work presented earlier. Feng and associates used the transgenic *Thy1-YFP* mouse line, in which it is presumed that all RGC types are sparsely labeled, to examine the morphological changes of ON- and ON-OFF RGCs after IOP was elevated using laser photocoagulation (Feng et al., 2013). They found that the ON-RGCs were more susceptible than the ON-OFF RGCs in terms of dendritic field area. In an examination of SMI-32-positive ON cells (which were likely ON sustained αRGCs), dendritic branching was reduced. In a follow-up study, this group queried the functional properties of these broad classes of RGCs using a laser photocoagulation combined with microbead injection model and found that the receptive field sizes of ON- and OFF-RGCs decreased but not for ON-OFF RGCs (H. Chen et al., 2015). Furthermore, it appeared that there were fewer ON and OFF RGCs with large receptive field sizes, suggesting that large RGCs of these physiologically defined types may be more vulnerable after chronic IOP elevation. This latter finding is consistent with the work of previous investigators as detailed in section 1.1, which found that RGCs with larger somata may be selectively vulnerable to IOP elevation.

A more detailed structural and functional analysis of several different RGC types was performed using the microbead injection model in *Thy1-YFP* mice, which also chronically elevates IOP (Della Santina et al., 2013). Four classes of RGCs (OFF transient, OFF sustained, ON transient, and ON sustained) were examined physiologically and it was found that they behave differently to IOP elevation. All types of RGCs demonstrated decreased spontaneous activity except for ON transient RGCs. Notably, the OFF transient RGCs were the only type to exhibit decreased receptive field radius. In parallel to its functional status, OFF transient RGCs were also the only type to show a reduction in dendritic arbor size and complexity. Interestingly, all types examined morphologically revealed a decrease in excitatory synapses, which for the OFF sustained and ON transient RGCs occurred prior to any change in dendritic area or complexity. This work also made important observations about the timing of functional and structural perturbations of RGCs in experimental glaucoma, which will be discussed in greater detail in Section 4.

While experimental models of glaucoma in which IOP elevation is chronic are considered more true to the chronic condition of human glaucoma, in practice many patients with glaucoma continue to progress even when IOP is controlled (Ederer et al., 2004; Heijl et al., 2002). Therefore, a mouse model in which IOP elevation is transient is very useful in understanding pathologic degenerative changes in RGCs that occur even once IOP is normalized, or in modeling acute angle closure crisis or intermittent angle closure, wherein the IOP is typically transiently elevated. We modified a laser-induced ocular hypertension model (Fu and Sretavan, 2010; Salinas-Navarro et al., 2009) in order to transiently elevate IOP in albino CD-1 mice, with return to baseline levels 7 days after laser. We examined rates of cell death using SMI-32 immunolabeling to identify αRGCs and found that the proportion of OFF transient RGCs lost was greater than for ON sustained RGCs (Ou et al., 2015). Using multielectrode array recordings, we measured ganglion cell responses, specifically ON sustained, ON transient, OFF sustained, and OFF transient RGCs. OFF

transient RGCs showed the earliest decrease in spontaneous activity at 14 days after laser, while ON transient RGCs did not have any alteration in spontaneous activity. Furthermore, as in the microbead occlusion model, only the OFF transient RGCs exhibited a decrease in receptive field size.

What about structural alterations that may correspond with functional changes? Certainly, one might expect that if OFF transient RGCs have reduced receptive field size that they would also have decreased dendritic area. Using a gene gun labeling technique in which individual RGCs are transfected with *CMV:tdTomato* and *CMV:PSD95-YFP*, we are able to distinguish between OFF and ON αRGCs in part by examining dendritic stratification (Fig. 1). As early as 3 days after IOP elevation, it appears that dendritic field area has decreased in the OFF vs. ON αRGCs, with even more shrinkage at 30 days after laser-induced ocular hypertension. There is also concomitant decrease in PSD95 puncta in the OFF αRGCs, whereas the complement of excitatory synapses appears relatively intact in the ON αRGCs. In summary, OFF αRGCs, and specifically the OFF transient αRGC, appear more susceptible to IOP elevation as compared to the other αRGCs, supporting the hypothesis that there are likely RGC intrinsic and extrinsic factors that make one type more vulnerable than another. Furthermore, there is consistency of the specific type vulnerability across models of experimental glaucoma and different strains of mice, as the OFF transient αRGC was also found to be susceptible in a more chronic microbead occlusion model in pigmented mice (Della Santina et al., 2013).

Taking advantage of relatively newer transgenic mouse lines in which various RGC types are genetically labeled, El-Danaf and Huberman examined early changes to dendritic architecture 7 days after IOP elevation using the microbead injection model (El-Danaf and Huberman, 2015). The transgenic lines they chose to analyze included ones that labeled OFF transient αRGCs, On-Off directionally selective ganglion cells (DSGCs), On DSGCs and the M1 type of intrinsically photosensitive RGC (M1 ipRGC) (the latter type was identified by immunohistochemistry). As identified by Della Santina and colleagues, El-Danaf and Huberman found that the OFF transient αRGCs underwent the greatest reduction in dendritic length and area. In contrast, the On DSGCs were structurally unaffected 7 days after IOP elevation. Interestingly, when examining On-Off DSGCs, which have dendrites in both the ON and OFF sublamina of the inner plexiform layer, the length of the dendrites in the ON sublamina increased while in the OFF sublamina the length decreased. To take the analysis one step further, El Danaf and Huberman also examined M1 ipRGCs, which are functionally ON RGCs but stratify their dendrites in the OFF sublamina. While dendritic field area was unchanged, there was a reduction in dendritic complexity and length in the microbead injected eyes. These experiments suggested a general rule that RGCs with a majority of dendrites in the OFF sublamina undergo the greatest morphologic change, whereas RGCs with a majority of dendrites in the ON sublamina remain resistant to IOP elevation. Note that the structural data from Della Santina and colleagues is consistent with this finding.

Certainly, there is now emerging evidence of RGC type-dependent morphological and functional degenerative patterns in mouse models of experimental glaucoma. Furthermore, it is promising that there is some consensus with regards to which types are more susceptible

(Fig. 2), even though it is obviously very challenging to profile how each RGC type degenerates given the sheer number of different RGC types. The vulnerability of the OFF transient αRGC is most consistently identified across studies performed by different laboratories and across different mouse strains and models of experimental glaucoma (Della Santina et al., 2013; El-Danaf and Huberman, 2015; Ou et al., 2015). However, there are discrepancies among studies; for example the dendritic field area of ON sustained αRGCs was decreased in a study using the microbead occlusion model (Della Santina et al., 2013) but unchanged in a study using a laser model of chronic angle closure glaucoma (Feng et al., 2013) (Fig. 2). These discrepancies can likely be explained by different time points during which RGCs are examined, the extent of damage induced, or factors such as morphologic characteristics of certain RGC types varying depending on retinal location (Bleckert et al., 2014). Furthermore, each model, whether inducing IOP elevation in an acute or chronic fashion, as well as the magnitude of IOP elevation, likely damages RGCs on varying time scales and to varying degrees. Thus, while it is not surprising that there are different morphological and functional alterations identified across different studies, it is interesting that certain patterns appear to be emerging. El Danaf and Huberman hypothesize that RGCs with dendrites that stratify in the OFF sublamina of the IPL are the most vulnerable to IOP elevation, and several independent studies corroborate this hypothesis (Della Santina et al., 2013; Ou et al., 2015). The vulnerability of the OFF sublamina may be related to susceptibility to vascular damage, as suggested by El-Danaf and Huberman, or other cell-extrinsic factors such as gradients of protective neurotrophic factors or toxic mediators, or cell-intrinsic factors such as the expression of transient receptor potential vanilloid (TRPV) channels that can induce apoptosis (Ryskamp et al., 2011; Sappington et al., 2009). Identification of what makes one RGC type susceptible and another type relatively resistant is a line of investigation worth pursuing, and would yield potential novel diagnostic and therapeutic targets in the treatment of glaucoma.

An important question in understanding the implications of these investigations is how these animal models relate to human glaucoma. While the microbead injection model is a chronic model, the structural changes identified by the various groups above are quite early, as early as 7 days after IOP elevation to modest levels (El-Danaf and Huberman, 2015). In contrast, the laser-induced ocular hypertension model transiently elevates IOP to a higher level, and may more closely mimic an acute angle closure crisis or intermittent angle closure. Indeed, in primate models of experimental glaucoma, dendritic alterations were the earliest structural alterations identified, occurring as early as 2.5 weeks after IOP elevation (Weber et al., 1998). In the clinic, we follow the mantra that “structure precedes function,” because we are only able to detect optic nerve and retinal nerve fiber layer alterations prior to visual field defects. However, it is likely that functional decrements are among the earliest signs of RGC injury (Della Santina et al., 2013), but we do not yet have tests of high enough sensitivity to detect such early changes in humans. However, there is evidence that even a single episode of acute angle closure crisis can result in early and subsequent visual field deficits (Aung et al., 2001; Bonomi et al., 1999; Y.-J. Chen et al., 2012; Douglas et al., 1975; Sng et al., 2011). In addition, synaptic and dendritic degeneration, which likely occurs prior to significant retinal nerve fiber layer loss, is not yet detectable using current imaging technology in human glaucoma patients. A detailed correlation between functional

impairment and structural changes within specific types of human RGCs is currently missing, as well as methods to investigate these questions in glaucoma patients. Both structural and functional tests designed to detect earlier anatomic and functional alterations in human glaucoma are thus important areas on which to focus future translational research efforts.

## 4. Sequence of events in glaucomatous neurodegeneration and implications for treatment

While the variety of animal models in which experimental glaucoma has been studied has raised some conflicting data, it has also allowed us to identify a discrete cascade of events recurring across animal models, which likely represents milestone steps of the degeneration process happening within all RGC types (Fig. 3). Evidence supports the hypothesis that the process of RGC neurodegeneration may be compartmentalized at the subcellular level whereby independent degenerative pathways occur in the soma, axon, dendrite, and synapse (Morquette and Di Polo, 2008; Nickells et al., 2012; Whitmore et al., 2005). Based on our investigations as well as the investigations of others, we hypothesize the following sequence of events in RGC glaucomatous degeneration, summarized schematically in Fig. 3. Following IOP elevation of varying duration and amplitude, this insult initially affects the axonal compartment of the cell at the lamina cribrosa of the optic nerve head, resulting in impaired axonal transport and initiation of the degeneration process. The next sign of RGC degeneration is an alteration in functional activity, especially a reduction in sensitivity to light (Della Santina et al., 2013; Ou et al., 2015; Pang et al., 2015). In parallel, there is a reduction of the excitatory synapses on the cell's dendritic arbor (Della Santina et al., 2013). Different RGC types enter this initial phase of degeneration at different time scales, generating a first differential susceptibility factor that is specific to RGC type. The next step is a reduction of the RGC's functional receptive field size, paralleled by a reduction in size and complexity of the cell's dendritic arbor. Disconnection from presynaptic partners likely plays an important role in the late phase of degeneration with the induction of secondary alterations in bipolar cells and photoreceptors, as indicated by the occurrence of ERG alterations in the late phase of several experimental glaucoma models (Aihara et al., 2003; Bayer et al., 2001; H. Chen et al., 2015). Once RGCs are disconnected from their synaptic partners and their function is compromised, cell death is triggered by apoptosis (Qu et al., 2010).

The two major caveats to directly confirm a universal sequence of degenerative events are the difficulty of longitudinally observing the degeneration process of individual RGCs at the level of structural detail desired (e.g. synaptic, dendritic, and axonal compartments), and the difficulty of tracking a specific RGC circuit with its pre- and post-synaptic partners in the live animal. Recent transgenic and virus-mediated circuit tracking (Duan et al., 2015) is putting this ambitious goal within reach. Still unknown are the intracellular events that transition each event of this sequence into the next one, as a specific transcriptomic and proteomic analysis needs to be carried out at the correct time point for each cell type. Although this task is very challenging to accomplish it can potentially lead to the

identification of multiple therapeutic targets to slow down and possibly interrupt the degenerative process.

While IOP is the only modifiable risk factor in glaucoma and indeed our only treatment option, the studies discussed in this review highlight the potential for rescuing RGCs if timed early enough in the degenerative sequence before major structural and functional alterations occur. Indeed, there is growing evidence that targeting the classical pathway of the complement cascade could be a promising avenue for slowing dendritic and synaptic degeneration of RGCs (Stevens et al., 2007; Williams et al., 2016). We also highlight the importance of further research to understand the mechanisms and principles that may govern RGC type selective vulnerability in glaucomatous neurodegeneration. From the perspective of optic nerve regeneration, there is now also emerging evidence demonstrating that different RGC types are more or less capable of axonal regeneration after optic nerve crush injury (Duan et al., 2015). If we could identify a unique type or several types of RGCs that are particularly susceptible or resistant to IOP elevation, or especially robust at axonal regeneration, we could better design improved functional tests and neuroprotective, neuroenhancing, or neuroregenerating treatments for glaucoma.

## 5. Dedication: David L. Epstein

This article is dedicated to David L. Epstein for his genuine, giving, and impactful mentorship and friendship, as well as his support of my development as a clinician-scientist, along with countless other mentees. I first met Dr. Epstein when interviewing for glaucoma fellowship at Duke, and there he asked a group of us what was the greatest challenge to departments of ophthalmology. His answer was the erosive segregation of ophthalmology from other departments in the university, and his desire to cross disciplines, innovate, and collaborate is a common thread throughout his career and informed his leadership philosophy. Dr. Epstein’s passion for studying the trabecular meshwork and developing treatments that targeted this diseased tissue has moved the field vertically in terms of our understanding of glaucoma pathogenesis and our ability to help patients, and continues to be carried forward by many of the contributors to this special issue. However, he also acknowledged that irrespective of trabecular disease, efforts should also be focused on preventing and reversing glaucomatous optic nerve damage. It is my hope to always honor his memory by carrying forward his teachings and cultivating my inquisitiveness both in my “clinical” and neuroscience laboratory (and pass these lessons onto mentees!) in order to translate science into better diagnostics and therapeutics that help our glaucoma patients now and in the future.

Yvonne Ou, San Francisco, California.

## Acknowledgments

This work was supported in part by NIH Grant KEY022676A, Research to Prevent Blindness Career Development Award, American Glaucoma Society Young Clinician-Scientist Award, Ron and Anita Wornick and That Man May See, NIH-NEI EY002162 Core Grant for Vision Research, and a Research to Prevent Blindness Unrestricted Grant to the University of California, San Francisco.

## References


Aihara M, Lindsey JD, Weinreb RN. Experimental mouse ocular hypertension: establishment of the model. Invest Ophthalmol Vis Sci. 2003; 44:4314–4320. [PubMed: 14507875]

Aung T, Looi AL, Chew PT. The visual field following acute primary angle closure. Acta Ophthalmol Scand. 2001; 79:298–300. [PubMed: 11401643]

Baden, T., Berens, P., Franke, K., Roman, Roson M., Bethge, M., Euler, T. The functional diversity of retinal ganglion cells in the mouse. Nature. 2016. http://dx.doi.org/10.1038/nature16468

Bayer AU, Neuhardt T, May AC, Martus P, Maag KP, Brodie S, Lütjen-Drecoll E, Podos SM, Mittag T. Retinal morphology and ERG response in the DBA/2NNia mouse model of angle-closure glaucoma. Invest Ophthalmol Vis Sci. 2001; 42:1258–1265. [PubMed: 11328737]

Bleckert A, Schwartz GW, Turner MH, Rieke F, Wong ROL. Visual space is represented by nonmatching topographies of distinct mouse retinal ganglion cell types. Curr Biol. 2014; 24:310–315. http://dx.doi.org/10.1016/j.cub.2013.12.020. [PubMed: 24440397]

Bonomi L, Marraffa M, Marchini G, Canali N. Perimetric defects after a single acute angle-closure glaucoma attack. Graefes Arch Clin Exp Ophthalmol. 1999; 237:908–914. [PubMed: 10541901]

Chaturvedi N, Hedley-Whyte ET, Dreyer EB. Lateral geniculate nucleus in glaucoma. Am J Ophthalmol. 1993; 116:182–188. [PubMed: 8352303]

Chen H, Zhao Y, Liu M, Feng L, Puyang Z, Yi J, Liang P, Zhang HF, Cang J, Troy JB, Liu X. Progressive degeneration of retinal and superior collicular functions in mice with sustained ocular hypertension. Invest Ophthalmol Vis Sci. 2015; 56:1971–1984. http://dx.doi.org/10.1167/iovs.14-15691. [PubMed: 25722210]

Chen YJ, Tai MC, Cheng JH, Chen JT, Chen YH, Lu DW. The longitudinal changes of the visual field in an Asian population with primary angle-closure glaucoma with and without an acute attack. J Ocul Pharmacol Ther. 2012; 28:529–535. http://dx.doi.org/10.1089/jop.2012.0006. [PubMed: 22690869]

Cleland BG, Levick WR, Wässle H. Physiological identification of a morphological class of cat retinal ganglion cells. J Physiol (Lond). 1975; 248:151–171. [PubMed: 1151804]

Dandona L, Hendrickson A, Quigley HA. Selective effects of experimental glaucoma on axonal transport by retinal ganglion cells to the dorsal lateral geniculate nucleus. Invest Ophthalmol Vis Sci. 1991; 32:1593–1599. [PubMed: 1707861]

Della Santina L, Inman DM, Lupien CB, Horner PJ, Wong ROL. Differential progression of structural and functional alterations in distinct retinal ganglion cell types in a mouse model of glaucoma. J Neurosci. 2013; 33:17444–17457. http://dx.doi.org/10.1523/JNEUROSCI.5461-12.2013. [PubMed: 24174678]

Douglas GR, Drance SM, Schulzer M. The visual field and nerve head in angle-closure glaucoma. a comparison of the effects of acute and chronic angle closure. Arch Ophthalmol. 1975; 93:409–411. [PubMed: 1131080]

Duan X, Qiao M, Bei F, Kim IJ, He Z, Sanes JR. Subtype-specific regeneration of retinal ganglion cells following axotomy: effects of osteopontin and mTOR signaling. Neuron. 2015; 85:1244–1256. http://dx.doi.org/10.1016/j.neuron.2015.02.017. [PubMed: 25754821]

Ederer F, Gaasterland DA, Dally LG, Kim J, VanVeldhuisen PC, Blackwell B, Prum B, Shafranov G, Allen RC, Beck A, Investigators, A.G.I.S. The Advanced glaucoma intervention study (AGIS): 13. comparison of treatment outcomes within race: 10-year results. Ophthalmology. 2004; 111:651–664. http://dx.doi.org/10.1016/j.ophtha.2003.09.025. [PubMed: 15051195]

El-Danaf RN, Huberman AD. Characteristic patterns of dendritic remodeling in early-stage glaucoma: evidence from genetically identified retinal ganglion cell types. J Neurosci. 2015; 35:2329–2343. http://dx.doi.org/10.1523/JNEUROSCI.1419-14.2015. [PubMed: 25673829]

Epstein DL. Open angle glaucoma. Why not a cure? Arch Ophthalmol. 1987; 105:1187–1188. [PubMed: 3632438]

Epstein, DL., Allingham, RR., Shuman, JS. Chandler and Grant's Glaucoma. fourth. Lippincott Williams & Wilkins; Baltimore: 1997.

Feng L, Zhao Y, Yoshida M, Chen H, Yang JF, Kim TS, Cang J, Troy JB, Liu X. Sustained ocular hypertension induces dendritic degeneration of mouse retinal ganglion cells that depends on cell

type and location. Invest Ophthalmol Vis Sci. 2013; 54:1106–1117. http://dx.doi.org/10.1167/iovs.12-10791. [PubMed: 23322576]

Fu CT, Sretavan D. Laser-induced ocular hypertension in albino CD-1 mice. Invest Ophthalmol Vis Sci. 2010; 51:980–990. http://dx.doi.org/10.1167/iovs.09-4324. [PubMed: 19815738]

Glovinsky Y, Quigley HA, Dunkelberger GR. Retinal ganglion cell loss is size dependent in experimental glaucoma. Invest Ophthalmol Vis Sci. 1991; 32:484–491. [PubMed: 2001923]

Heijl A, Leske MC, Bengtsson B, Hyman L, Bengtsson B, Hussein M, Early Manifest. Glaucoma Trial Group. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. Arch Ophthalmol. 2002; 120:1268–1279. [PubMed: 12365904]

Jakobs TC, Libby RT, Ben Y, John SWM, Masland RH. Retinal ganglion cell degeneration is topological but not cell type specific in DBA/2J mice. J Cell Biol. 2005; 171:313–325. http://dx.doi.org/10.1083/jcb.200506099. [PubMed: 16247030]

Kerrigan-Baumrind LA, Quigley HA, Pease ME, Kerrigan DF, Mitchell RS. Number of ganglion cells in glaucoma eyes compared with threshold visual field tests in the same persons. Invest Ophthalmol Vis Sci. 2000; 41:741–748. [PubMed: 10711689]

Li RS, Chen BY, Tay DK, Chan HHL, Pu ML, So KF. Melanopsin-expressing retinal ganglion cells are more injury-resistant in a chronic ocular hypertension model. Invest Ophthalmol Vis Sci. 2006; 47:2951–2958. http://dx.doi.org/10.1167/iovs.05-1295. [PubMed: 16799038]

McKendrick AM, Sampson GP, Walland MJ, Badcock DR. Contrast sensitivity changes due to glaucoma and normal aging: low-spatial-frequency losses in both magnocellular and parvocellular pathways. Invest Ophthalmol Vis Sci. 2007; 48:2115–2122. http://dx.doi.org/10.1167/iovs.06-1208. [PubMed: 17460269]

Morgan JE. Selective cell death in glaucoma: does it really occur? Br J Ophthalmol. 1994; 78:875–879. discussion 879–80. [PubMed: 7848987]

Morquette JB, Di Polo A. Dendritic and synaptic protection: is it enough to save the retinal ganglion cell body and axon? J Neuroophthalmol. 2008; 28:144–154. http://dx.doi.org/10.1097/WNO.0b013e318177edf0. [PubMed: 18562848]

Nelson R, Famiglietti EV, Kolb H. Intracellular staining reveals different levels of stratification for on- and off-center ganglion cells in cat retina. J Neurophysiol. 1978; 41:472–483. [PubMed: 650277]

Nickells RW, Howell GR, Soto I, John SWM. Under pressure: cellular and molecular responses during glaucoma, a common neurodegeneration with axonopathy. Annu Rev Neurosci. 2012; 35:153–179. http://dx.doi.org/10.1146/annurev.neuro.051508.135728. [PubMed: 22524788]

Ou Y, Chu K, Ullian EM, Wong RO, Della Santina L. Subtype specific retinal ganglion cell dysfunction in a mouse model of ocular hypertension. Presented at the ARVO annual meeting. Invest Ophthalmol Vis Sci. 2015; 2443

Pang JJ, Gao F, Wu SM. Light-evoked excitatory and inhibitory synaptic inputs to ON and OFF alpha ganglion cells in the mouse retina. J Neurosci. 2003; 23:6063–6073. [PubMed: 12853425]

Pang JJ, Frankfort BJ, Gross RL, Wu SM. Elevated intraocular pressure decreases response sensitivity of inner retinal neurons in experimental glaucoma mice. Proc Natl Acad Sci U S A. 2015; 112:2593–2598. http://dx.doi.org/10.1073/pnas.1419921112. [PubMed: 25675503]

Qu J, Wang D, Grosskreutz CL. Mechanisms of retinal ganglion cell injury and defense in glaucoma. Exp Eye Res. 2010; 91:48–53. http://dx.doi.org/10.1016/j.exer.2010.04.002. [PubMed: 20394744]

Quigley HA. Neuronal death in glaucoma. Prog Retin Eye Res. 1999; 18:39–57. [PubMed: 9920498]

Quigley HA, Dunkelberger GR, Green WR. Chronic human glaucoma causing selectively greater loss of large optic nerve fibers. Ophthalmology. 1988; 95:357–363. [PubMed: 3174003]

Quigley HA, Sanchez RM, Dunkelberger GR, L'Hernault NL, Baginski TA. Chronic glaucoma selectively damages large optic nerve fibers. Invest Ophthalmol Vis Sci. 1987; 28:913–920. [PubMed: 3583630]

Ryskamp DA, Witkovsky P, Barabas P, Huang W, Koehler C, Akimov NP, Lee SH, Chauhan S, Xing W, Rentería RC, Liedtke W, Krizaj D. The polymodal ion channel transient receptor potential vanilloid 4 modulates calcium flux, spiking rate, and apoptosis of mouse retinal ganglion cells. J Neurosci. 2011; 31:7089–7101. http://dx.doi.org/10.1523/JNEUROSCI.0359-11.2011. [PubMed: 21562271]

Salinas-Navarro M, Alarcon-Martínez L, Valiente-Soriano FJ, Ortín-Martínez A, Jimenez-Lopez M, Aviles-Trigueros M, Villegas-Perez MP, la Villa De P, Vidal-Sanz M. Functional and morphological effects of laser-induced ocular hypertension in retinas of adult albino Swiss mice. Mol Vis. 2009; 15:2578–2598. [PubMed: 20011633]

Sample PA. What does functional testing tell us about optic nerve damage? Surv Ophthalmol. 2001; 45(Suppl. 3):S319–S324. discussion S332–4. [PubMed: 11377456]

Sanes JR, Masland RH. The types of retinal ganglion cells: current status and implications for neuronal classification. Annu Rev Neurosci. 2015; 38:221–246. http://dx.doi.org/10.1146/annurev-neuro-071714-034120. [PubMed: 25897874]

Sappington RM, Sidorova T, Long DJ, Calkins DJ. TRPV1: contribution to retinal ganglion cell apoptosis and increased intracellular Ca2+ with exposure to hydrostatic pressure. Invest Ophthalmol Vis Sci. 2009; 50:717–728. http://dx.doi.org/10.1167/iovs.08-2321. [PubMed: 18952924]

Saxena S, Caroni P. Selective neuronal vulnerability in neurodegenerative diseases: from stressor thresholds to degeneration. Neuron. 2011; 71:35–48. http://dx.doi.org/10.1016/j.neuron.2011.06.031. [PubMed: 21745636]

Sng CCA, See JSL, Ngo CS, Singh M, Chan YH, Aquino MC, Tan AM, Shabana N, Chew PTK. Changes in retinal nerve fibre layer, optic nerve head morphology, and visual field after acute primary angle closure. Eye (Lond). 2011; 25:619–625. http://dx.doi.org/10.1038/eye.2011.31. [PubMed: 21436844]

Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, Sher A, Litke AM, Lambris JD, Smith SJ, John SWM, Barres BA. The classical complement cascade mediates CNS synapse elimination. Cell. 2007; 131:1164–1178. http://dx.doi.org/10.1016/j.cell.2007.10.036. [PubMed: 18083105]

Sümbül U, Song S, McCulloch K, Becker M, Lin B, Sanes JR, Masland RH, Seung HS. A genetic and computational approach to structurally classify neuronal types. Nat Commun. 2014; 5:3512. http://dx.doi.org/10.1038/ncomms4512. [PubMed: 24662602]

Vickers JC, Schumer RA, Podos SM, Wang RF, Riederer BM, Morrison JH. Differential vulnerability of neurochemically identified subpopulations of retinal neurons in a monkey model of glaucoma. Brain Res. 1995; 680:23–35. [PubMed: 7663981]

Wässle H, Peichl L, Boycott BB. Morphology and topography of on- and off-alpha cells in the cat retina. Proc R Soc Lond, B, Biol Sci. 1981; 212:157–175. [PubMed: 6166012]

Weber AJ, Chen H, Hubbard WC, Kaufman PL. Experimental glaucoma and cell size, density, and number in the primate lateral geniculate nucleus. Invest Ophthalmol Vis Sci. 2000; 41:1370–1379. [PubMed: 10798652]

Weber AJ, Kaufman PL, Hubbard WC. Morphology of single ganglion cells in the glaucomatous primate retina. Invest Ophthalmol Vis Sci. 1998; 39:2304–2320. [PubMed: 9804139]

Whitmore AV, Libby RT, John SWM. Glaucoma: thinking in new ways-a rôle for autonomous axonal self-destruction and other compartmentalised processes? Prog Retin Eye Res. 2005; 24:639–662. http://dx.doi.org/10.1016/j.preteyeres.2005.04.004. [PubMed: 15953750]

Williams PA, Tribble JR, Pepper KW, Cross SD, Morgan BP, Morgan JE, John SWM, Howell GR. Inhibition of the classical pathway of the complement cascade prevents early dendritic and synaptic degeneration in glaucoma. Mol Neurodegener. 2016; 11:26. http://dx.doi.org/10.1186/s13024-016-0091-6. [PubMed: 27048300]

Zhang P, Wen W, Sun X, He S. Selective reduction of fMRI responses to transient achromatic stimuli in the magnocellular layers of the LGN and the superficial layer of the SC of early glaucoma patients. Hum Brain Mapp. 2016; 37:558–569. http://dx.doi.org/10.1002/hbm.23049. [PubMed: 26526339]

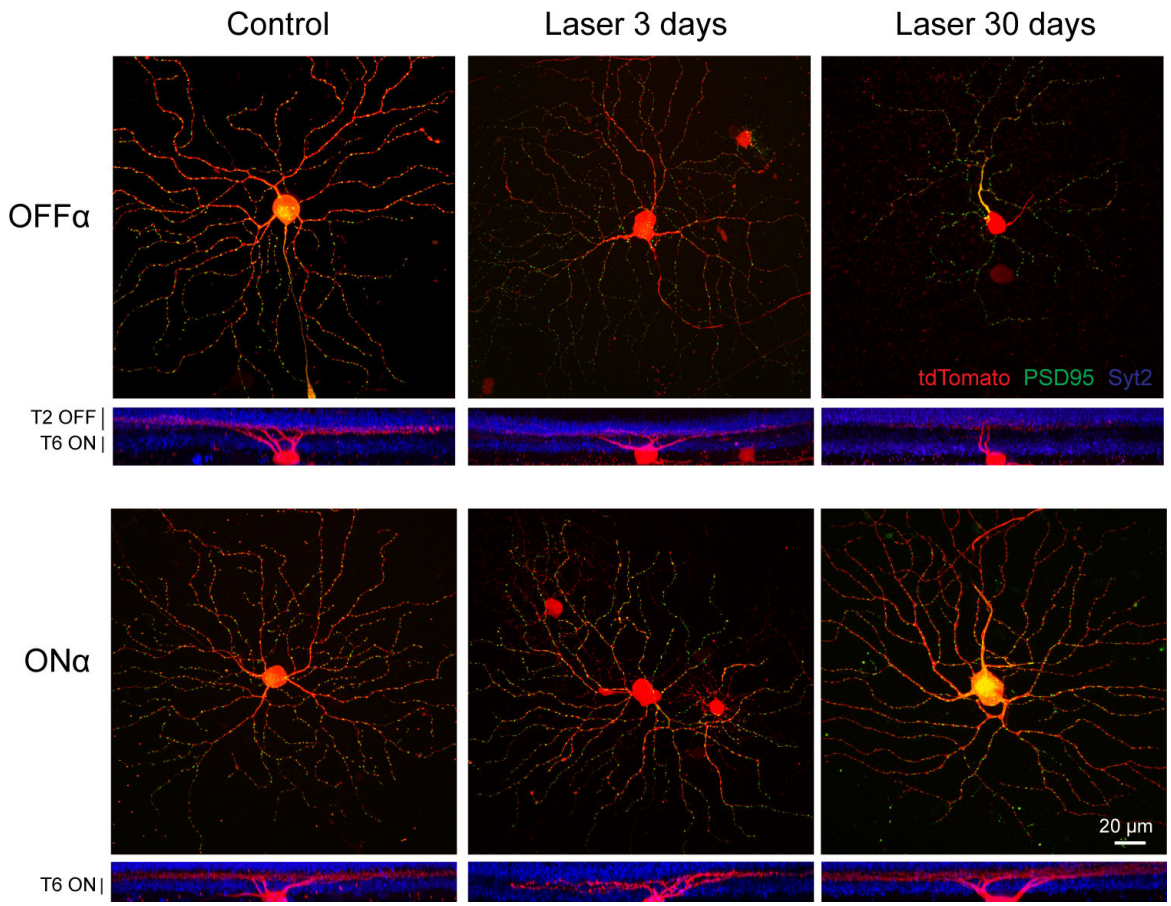


**Fig. 1.**

Differential dendritic shrinkage and loss of synapses between ON and OFF αRGCs after intraocular pressure elevation. Biolistically labeled alpha RGCs (αRGCs) in control retinas (first column) and 3 or 30 days after laser-induced ocular hypertension in mice (second and third column, respectively). Cell morphology was visualized by cytosolic transfection of tdTomato (red), while excitatory post-synaptic sites on dendrites were labeled by co-transfection of the cell with PSD95-YFP (green). Dendritic stratification was visualized in the orthogonal views by immunolabeling against Synaptotagmin 2 (Syt2), a marker of Type 2 (T2) OFF and Type 6 (T6) ON bipolar cells. Despite similar dendritic arbor size in control retinas, OFF αRGCs undergo greater dendritic and synaptic degeneration compared to ON αRGCs at both 3 and 30 days after intraocular pressure elevation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

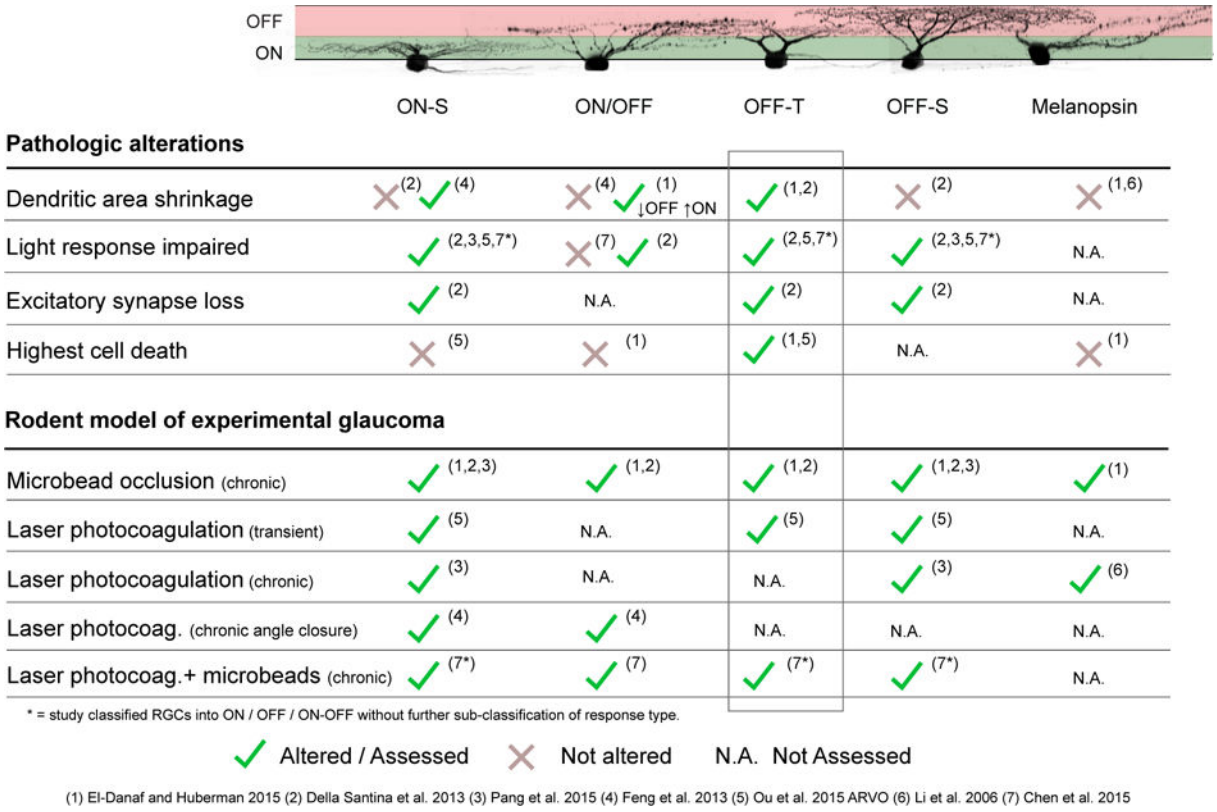


**Fig. 2.**

Summary of RGC pathologic alterations in experimental glaucoma in mice. Summary of the structural and physiological alterations (top table) observed in mouse alpha (ON-S, OFF-T, OFF-S), ON-OFF, and melanopsin-expressing RGCs in different models of experimental glaucoma. The lower table summarizes the RGC types examined in each experimental model. Top row: side-view examples of different RGC types biolistically labeled by tdTomato expression in the adult mouse retina. ON-S: ON sustained; OFF-T: OFF transient; OFF-S: OFF sustained.

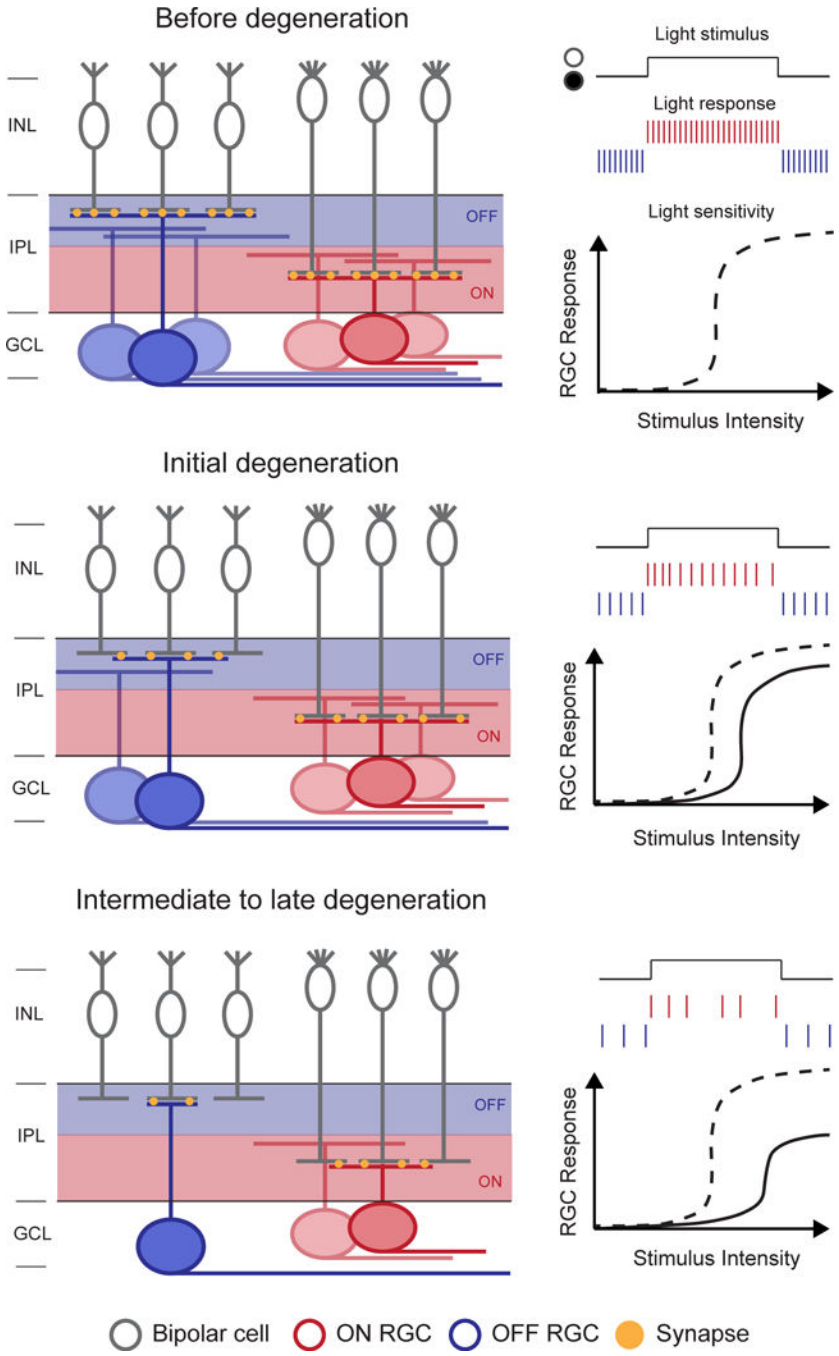


**Fig. 3.**

Common degenerative events leading to RGC death in experimental glaucoma. Schematic representation of the morphological and functional alterations of OFF vs. ON RGCs at different stages of glaucomatous neurodegeneration. Although occurring on different time scales, ON and OFF cells (red and blue, respectively) experience initial synapse loss paralleled by reduction of light response and sensitivity (right plots), followed by dendritic shrinkage and reduction of the functional receptive field. Eventually there is RGC cell death, with earlier loss of OFF RGCs followed by loss of ON RGCs. INL: inner nuclear layer; IPL:

inner plexiform layer; GCL: ganglion cell layer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)