Chapter 22
Dendrite Degeneration in Glaucoma

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Abstract Glaucoma is a group of slowly progressive neurodegenerative optic neuropathies in which the final common pathway is death of the retinal ganglion cells (RGCs) that comprise the optic nerve. While many risk factors have been identified, including intraocular pressure, the mechanistic pathway between the initial insult and RGC loss is still not clearly delineated. One paradigm is that of compartmentalized neurodegeneration, in which the axons, dendrites, and synapses undergo independent degenerative programs prior to cell soma loss. This chapter will focus on the features of dendritic remodeling in glaucoma and the implications on RGC function. Mounting evidence from various animal models of glaucoma demonstrates that changes in dendritic architecture precede cell death, although the detailed mechanisms and consequences of these morphologic and functional alterations have yet to be fully dissected. Such early dendritic alterations and synaptic rearrangements influence the normal function and structure of RGCs, thus giving important insight into the mechanism of circuit disassembly. At the same time, imaging of early dendritic alterations and identification of specific features of visual function that are first impaired represent promising new approaches to earlier diagnosis of this disease and improved disease progression recognition in humans.

Keywords Glaucoma • Dendrite • Neurodegeneration • Retinal ganglion cell • Dendritic remodeling

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22.1 The Neurodegenerative Process in Glaucoma

Glaucoma comprises a group of disorders characterized by progressive neurodegeneration and eventual loss of the retinal ganglion cells (RGCs) that compose the optic nerve and project to central visual targets in the brain. It is the leading cause of irreversible blindness worldwide, estimated to affect 70 million people (Quigley and Broman 2006). One of the major risk factors of glaucoma is intraocular pressure (IOP), although it is apparent that elevated IOP is neither necessary nor sufficient for either disease diagnosis or progression. However, reduction of IOP remains the mainstay of medical and surgical treatment of glaucoma, even for patients in whom the IOP is considered to be in the normal range (Collaborative Normal-Tension Glaucoma Study Group 1998). Much research has been focused on the pathologic sequence of degeneration in order to better understand the mechanism by which IOP or other factors results in loss of RGCs.

A major gap in our diagnostic and treatment paradigms for glaucoma is that they are often initiated after there is already evidence of RGC death and visual field loss. There is growing consensus that the site of initial injury is the RGC axon as it passes through the pores of the lamina cribrosa at the optic nerve head (Quigley 1999; Quigley et al. 2000; Howell et al. 2007). Therefore, focal axonal injury is thought to be a key component of RGC glaucomatous pathology. Eventually, the neuronal soma undergoes apoptosis and irreversible RGC death occurs. However, our understanding of the pathological cellular processes that occur between initial injury and eventual apoptotic cell death is incomplete. Emerging evidence supports the hypothesis that the pathological degeneration of a stressed RGC may be compartmentalized at the subcellular level (Whitmore et al. 2005; Morquette and Di Polo 2008; Nickells et al. 2012). There may be independent degenerative pathways in the soma, axon, dendrite, and synapse. Indeed, compartmentalized neurodegeneration in which the axons, dendrites, and synapses degenerate independently of the soma occurs in other brain neurodegenerative diseases and may develop prior to late clinical signs (Raff et al. 2002; Wishart et al. 2006). Thus, it is not surprising to find that recent attention has focused on the cellular events at distal synapses and dendritic morphologic changes that precede neuronal death. This chapter focuses on the dendritic architecture of RGCs in the context of glaucoma and the functional implication of dendritic remodeling in glaucoma pathogenesis.

22.2 Imaging Dendrite Degeneration

Before delving into a discussion of the dendritic changes in glaucoma, we will summarize the techniques used to assess RGC morphology. Imaging of RGCs undergoing degeneration in glaucoma has been performed in multiple animal species but still represents a technical challenge because of the detail needed to

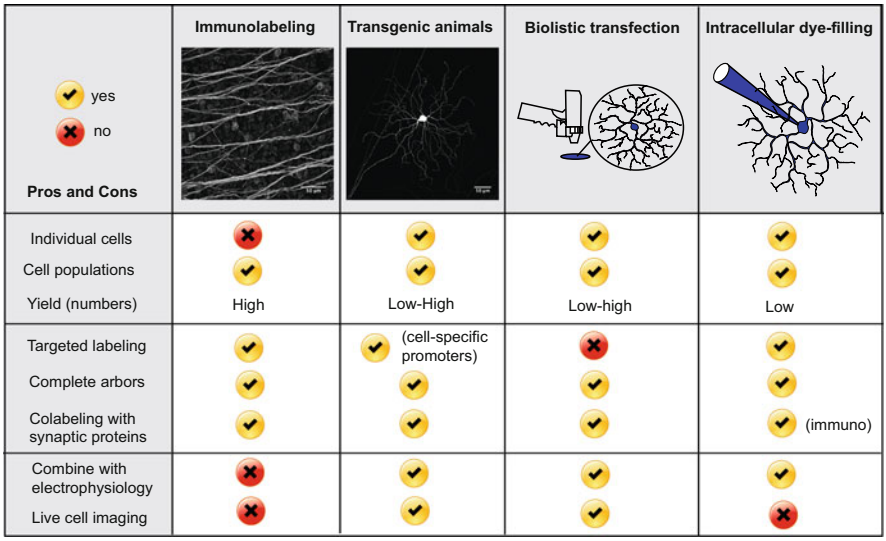


Fig. 22.1 Methods for labeling RGCs: advantages and limitations. Visualization of retinal ganglion cells (RGCs) in experimental glaucoma is critical to imaging and evaluating changes in morphology that could be related to functional alterations. Shown here are various techniques to label RGCs, together with their advantages and limitations. Immunolabeling can reveal RGC morphology with reasonable detail, but it is difficult to label individual cells with this method. Moreover, it cannot be combined with electrophysiology although it is possible to immunolabel cells after physiological recordings. Transgenic animals in which subpopulations of RGCs are labeled by fluorescent protein expression have many advantages, including labeling of specific RGC types, live cell imaging, and targeting RGCs for electrophysiological recording. Biolistic transfection to cause expression of fluorescent protein in RGCs also has similar advantages, but cells are randomly labeled. Finally, intracellular dye filling allows for simultaneous visualization of the cell and recording of the cell's responses, but time-lapse cell imaging is not possible

catch subtle or early changes in dendritic architecture. A summary of common methods used to label RGCs, along with their advantages and disadvantages, is illustrated in Fig. 22.1.

Most of the early imaging techniques were restricted to the use of Golgi staining, antibody labeling, and intracellular dye filling of RGCs (Weber et al. 1998), mainly in primates and cats. These techniques, which allow visualization of RGC dendrite morphology in fixed tissues, were usually applied to investigate late stages of the degenerative process. These approaches successfully identified key changes in dendritic architecture such as shrinkage of the dendritic arbor (Shou et al. 2003; Weber and Harman 2005). Concomitant labeling of the entire cell also revealed the relationship between dendritic alterations occurring in the axon and cell body. However, these labeling techniques exhibited several key limitations. For example, antibody labeling is limited by the relative low availability of antisera able to specifically reveal the dendritic morphology of RGCs. Frequently, investigators use antibodies against a generic component of the cytoskeleton, such as neurofilaments (Straznicky et al. 1992), which label multiple RGC types and

therefore makes it difficult to identify the morphology of individual cells (Fig. 22.1). In addition, labeling cytoskeletal components usually fails to reveal the fine structure of the distal dendrites of the ganglion cell, leading to underestimates of dendritic field size. Intracellular dye injection enables targeting RGCs based on their soma size, a method useful, for example, in differentiating alpha RGCs with their relatively large somata. Another major strength of this technique is that it can be performed together with recording of electrophysiological properties, allowing a thorough characterization of both functional characteristics and dendritic morphology of the cell.

Effective labeling of RGC morphology can also be attained by injection of retrogradely transported dyes at the level of axonal terminals of RGCs (Vidal-Sanz et al. 1988; Dacey et al. 2003). The amount of labeled cells varies from a few cells to almost the entire population of RGCs depending on the amount of dye injection and the elapsed time between dye injection and tissue collection. This technique can be used to label RGCs in various species and all types of RGCs but relies on the presence of intact axons and their retrograde transport mechanisms.

Transgenic mice in which RGCs express fluorescent proteins have greatly facilitated imaging and comparison of RGC dendritic arbors in mouse models of glaucoma. For example, the dendritic arbor is labeled completely in *Thy1-YFP* mouse lines, and in some lines, sparse yellow fluorescent protein (YFP) expression enables the dendritic arbors of isolated RGCs to be distinguished from that of their neighbors (Feng et al. 2000). An alternative approach to obtain single-cell resolution for imaging RGC dendritic arbors is to use a biolistic method to acutely transfect retinas with plasmids encoding a fluorescent protein (Koizumi et al. 2011; Morgan et al. 2011; Della Santina et al. 2013). This "gene-gun" delivery method results in random labeling of RGCs with fluorescent proteins. Recently, the creation of transgenic mice expressing fluorescent protein in specific types of RGCs (Huberman et al. 2008, 2009; Dhande et al. 2013) permitted investigation of glaucoma-induced alterations in the dendrites of genetically identified RGC populations (El-Danaf and Huberman 2015).

A step beyond the analysis of the dendritic morphologic defects of RGCs in glaucoma is the investigation of the synapses between RGCs in the diseased retina and their presynaptic partners. Immunolabeling against presynaptic or postsynaptic proteins permits quantification of the density of excitatory synapses in the entire neuropil upon IOP elevation. However, in order to attain the density and distribution of synapses along individual RGC dendritic arbors, investigators have used biolistic methods to co-label dendrites with a fluorescent protein and excitatory postsynaptic sites with the postsynaptic protein, PSD95 (Morgan et al. 2008), tagged with a fluorescent protein of a different color.

Despite recent attempts to use *Thy1-FP* transgenic mice for imaging RGC death in vivo (Raymond et al. 2009) as well as their dendritic structure (Walsh and Quigley 2008), only a semiquantitative appreciation of the dendritic degeneration process in optic neuropathies has been possible thus far. Current limitations are dictated mainly by the difficulty in obtaining high-resolution in vivo images of RGC dendrites in the eyes of pigmented animals, where the imaging path must pass

through the cornea and lens of the animal. Progress in longitudinal imaging of dendritic structure has been accomplished in rodents in optic nerve crush and retinal ischemia models, where the cornea remains clear (Leung et al. 2008a, b, 2011; Li et al. 2011). Using confocal scanning laser ophthalmoscopy (cSLO), Leung et al. demonstrated dendritic shrinkage as the earliest morphologic change after optic nerve crush using *Thy1-FP* mice. This technique does not permit evaluation of different RGC types since *en face* live imaging does not provide dendritic stratification information and is likely further limited by imaging brightly labeled dendritic arbors in the inner half of the IPL. Future improvement in cSLO optics such as the introduction of adaptive optics (Werkmeister et al. 2013) will hopefully bridge this technical gap. In order to extend the analysis of dendritic structure to humans, new methods need to be developed to label RGC dendrites. Current imaging techniques available in the clinic, such as cSLO or ocular coherence tomography (OCT), could then be used to follow the progression of dendritic alterations preceding cell death. One additional intriguing possibility is the use of spectral domain OCT (SD-OCT) to identify sublaminae within the IPL, which has recently been demonstrated using a commercially available SD-OCT instrument (Tanna et al. 2010).

22.3 Dendrite Remodeling in the Retina

Multiple lines of evidence in experimental glaucoma as well as human glaucoma suggest that dendrite remodeling occurs prior to cell death in response to intraocular pressure (IOP) elevation. One of the first studies to demonstrate that dendritic changes are an early marker of retinal ganglion cell degeneration was performed by intracellular labeling of individual RGCs in nonhuman primate retina (Weber et al. 1998). Using laser-induced scarification of the trabecular meshwork to chronically elevate IOP, Weber et al. examined 1298 intracellularly labeled RGCs and analyzed them both qualitatively and quantitatively in terms of soma size, dendritic field size, and axon diameter. This study demonstrated that the earliest measurable structural changes of RGC degeneration were dendritic in nature. The specific morphologic changes included thinning of proximal and distal dendrites, decreased dendritic process diameter at branch points, and reduced dendritic arbor complexity. Changes in axonal diameter occurred later, followed by smaller soma size. While animal models have provided many insights into the timing of RGC glaucomatous degeneration, human studies are much more rare given the difficulty in obtaining tissue and limitations in technical aspects of tissue preparation. One study using postmortem tissue of four patients with amaurotic (no light perception) glaucoma sheds some insight into dendritic changes in human retina (Pavlidis et al. 2003). Two of the four patients had a long-standing history of congenital glaucoma, while the remaining two had absolute secondary glaucoma with amaurosis and uncontrolled IOP greater than 40 mmHg. Although the RGC labeling technique using the fluorescent carbocyanine dye DiI has its limitations,

the authors estimated that less than 1 % of the stainable RGCs remained in the glaucomatous retinas. Therefore, the labeled cells that were examined were considered resistant to glaucoma. Nevertheless, these cells exhibited abnormal axonal beading and less dendritic complexity, despite the fact that the cell soma size was not appreciably shrunken. This data, although representative of only a subpopulation of human RGCs, demonstrates that dendritic shrinkage is indeed one of the earlier morphologic changes in RGC degeneration in glaucoma patients, consistent with the nonhuman primate model.

While dendrite remodeling is one of the earlier structural changes in response to elevated IOP, the susceptibility and extent of these changes vary among RGC types. For example, of particular interest to the investigators using the nonhuman primate model discussed above (Weber et al. 1998) was whether there would be differences between the midget and parasol cells, as there had been reports in the literature that elevated IOP differentially affected RGCs based on cell size (Johnson et al. 1989; Glovinsky et al. 1991, 1993; Vickers et al. 1995; Quigley 1998). Indeed, in this study, parasol cells showed a significant reduction in dendritic field size and axon diameter, suggesting a possible cell type-specific response dependent on dendritic field size. Consistent with the finding that cells with larger dendritic fields may be more susceptible in experimental glaucoma, Shou et al. used a feline model of chronic IOP elevation (via injection of endogenous ghost red blood cells into the anterior chamber of the eye) (Shou et al. 2003) to demonstrate that RGC loss and dendritic changes were more pronounced in the Y-type (large-field alpha cells) than the X-type (small-field beta cells) cells. The authors retrogradely labeled RGCs by bilateral injection of horseradish peroxidase into layers A and A1 of the lateral geniculate nucleus (LGN) and carefully analyzed the morphology of the labeled cells, including maximum dendrite radius, total length of dendrites, and number of dendritic bifurcations. All of the Y-type cells showed significantly greater shrinkage in dendritic structure parameters compared to the X-type cells.

However, more recently, multiple laboratories using various rodent models of glaucoma have found that there are RGC type-specific responses to IOP elevation that are not consistent with the hypothesis that only large-field RGCs are more susceptible. One of the first such studies used *Thy1-YFP* mice to investigate whether specific types of RGCs or RGCs from a specific location within the retina were differentially affected by elevated IOP in a laser-induced model of chronic ocular hypertension (Feng et al. 2013). In this study, the authors classified RGCs based on their dendrite ramification depth and studied a subset of them. ON RGC dendrites stratify in the inner half of the IPL and OFF dendrites ramify in the outer half of the IPL. ON and OFF RGCs respond best to increases and decreases in light intensity, respectively (Nelson et al. 1978). ON-OFF RGCs respond to both increment and decrement of light intensity and have bistratified dendritic arbors. Interestingly, this study revealed that there are type-specific and location-dependent RGC morphologic changes. Specifically, dendritic shrinkage began on the vertical axis with a subgroup of ON RGCs exhibiting smaller dendritic areas and shorter dendrites in the superior quadrant of glaucomatous retinas, in contrast to the nonhuman primate and cat studies, which showed that RGCs with larger dendritic areas are more

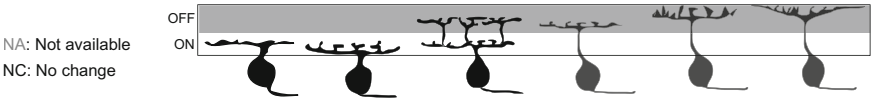
susceptible to IOP elevation (Weber et al. 1998; Shou et al. 2003). Furthermore, some RGCs had abnormal morphology, including dendrites that appeared to loop and fold back and dendritic trees pruned down to the primary dendrites. Consistent with other studies, dendritic degeneration preceded cell soma shrinkage. Although this data certainly supports the idea of RGC-type-specific dendritic morphologic changes in response to elevated IOP, only ON and ON-OFF RGCs could be analyzed in detail because there were few OFF RGCs labeled.

Not only does IOP elevation affect RGCs differentially depending on RGC type, but also distinct RGC types respond at different time scales and to varying degrees. Della Santina and colleagues investigated the morphologic and functional changes in various RGC types using the microbead injection technique to chronically elevate IOP in *Thy1-YFP* mice (Della Santina et al. 2013). This investigation focused its functional assessment on ON and OFF RGCs, specifically sustained or transient, depending on whether the RGC responds with a sustained or transient depolarization, and then focused the morphologic analysis on large-field alpha-like RGCs. OFF-transient RGCs were the most vulnerable to elevated IOP, exhibiting a more rapid decline in structural and functional parameters compared to OFF-sustained, ON-transient, and ON-sustained RGCs, with functional changes preceding the structural alterations. Indeed, when comparing these RGC types, only the OFF-transient RGCs showed a reduction in dendritic arbor size and complexity 30 days after IOP elevation. The distribution of excitatory postsynaptic sites along the dendritic arbor was also examined, and, as expected, OFF-transient RGCs with reduced dendritic arbor size and complexity had concomitant loss of excitatory postsynaptic sites. Interestingly, the OFF- and ON-sustained RGCs that had functional changes but no dendritic shrinkage also had loss of excitatory postsynaptic sites. Therefore, even before dendritic structure changes, the earliest biomarker of damage to RGCs is likely a loss of synapses.

Building on the hypothesis that dendritic alterations may be an early hallmark of RGC degeneration for specific RGC types, recent evidence suggests that the earliest dendritic remodeling events may occur in RGCs with dendrites that laminate in the OFF sublamina. El-Danaf and Huberman (2015) recently examined dendritic remodeling in genetically labeled RGCs using several different lines of mice after IOP elevation was induced by microbead injection. Furthermore, they examined these morphologic changes as early as 7 days after IOP elevation, highlighting the alterations that may be occurring in the earliest stages of disease. Consistent with the Della Santina study, El-Danaf and Huberman found that OFF-transient alpha RGCs (labeled in CB2-GFP mice) exhibited dendritic arbor changes, specifically decreased total dendritic length, albeit at a much earlier time point (7 days vs. 30 days after microbead injection). Furthermore, El-Danaf and Huberman noted that there was an asymmetric shrinkage of the dendritic arbors of these OFF-transient RGCs in a direction away from the temporal quadrant. Additional RGC types the authors examined using a genetically labeled line (Hoxd10-GFP) were the ON DSGCs (on direction-selective RGCs) and αON-OFF DSGCs. One week after IOP elevation, there was no apparent change in structural parameters in the ON DSGCs. In contrast, the αON-OFF DSGCs

exhibited no change in total dendritic length but had laminar alterations; specifically, the dendritic length of ON dendrites increased while the dendritic length of OFF dendrites decreased. To explore whether these laminar specific changes are driven by the general location of dendritic ramification or are specific to the functional characteristics of the RGC, the authors also examined the intrinsically photosensitive RGCs, the M1 ipRGCs. M1 ipRGCs are functionally ON-type RGCs, but their dendrites stratify deep within the IPL in the OFF sublamina. Surprisingly, while there was no change in soma size or dendritic field area between control and microbead injected retinas, there was a decrease in dendritic complexity and length in the M1 ipRGCs of IOP-elevated retinas. Although examination of these 4 RGC types is an incomplete evaluation given the likely 30 plus RGC types in the mouse retina (Sanes and Masland 2015), this work suggests that RGCs that stratify primarily in the ON sublamina of the IPL are relatively resistant to IOP elevation, whereas RGCs with dendrites in the OFF sublamina exhibit significant remodeling and loss. A summary of what is currently known about the morphologic and functional alterations that occur in different mouse RGC types in response to IOP elevation is provided in Fig. 22.2.

While it is apparent that RGCs undergo dendrite remodeling in response to IOP elevation in a type-specific manner, it is also true that these differences vary depending on the experimental animal model used. For example, as discussed above, Shou et al. showed that dendritic perturbations and RGC loss were more dramatic in cells of the Y-type (large-field alpha cells) than the X-type (small-field beta cells) in a chronic IOP elevation model (Shou et al. 2003). However, previous work by the same group using a feline model of acute IOP elevation with higher IOPs than the chronic model demonstrated that Y-type cells were actually more



Measurements	DS-ON	sON	DS-ON/OFF	tOFF	sOFF	Melanopsin M1
Number of cells (1)	↓	NA	↓	↓↓	NA	NC
Dendritic arbor size (1,2,4)	NC (1)	NC (2) ↓ (4)	NC (4) ON ↑ OFF ↓ (1)	↓ (2)	NC (2)	NC (1) (branching ↓)
Excitatory synapses (2)	NA	↓	NA	↓	↓	NA
Light response (2,3)	NA	↓ (2,3)	NA	↓ (2)	↓ (2,3)	NA
Cation/Cl^- currents (3)	NA	↓	NA	NA	↓	NA
Receptive field size (2,4)	NA	NC (2) ↓ (4)	NA	↓ (2)	NC (2)	NA

Fig. 22.2 Structural and functional alterations of distinct types of RGCs. Summary of the structural and functional changes in distinct retinal ganglion cell (RGC) types across different models of experimental glaucoma. Please refer to the text for a detailed explanation of the similarities and differences observed across studies. Summarized from (*1*) El-Danaf and Huberman 2015; (*2*) Della Santina et al. 2013; (*3*) Pang et al. 2015; (*4*) Feng et al. 2013

resistant to short-term IOP elevation than X-type cells (Shou and Zhou 1989), suggesting that different models of injury can induce different outcomes depending on cell type. These differences may reflect acute versus chronic IOP elevation, as well as the magnitude of IOP elevation, with higher IOPs resulting in retinal ischemia. As another example, Kalesnykas et al. (2012) examined soma size, neurite outgrowth, and dendritic complexity in both an optic nerve crush and the microbead injection glaucoma model using *Thy1-YFP* mice. Interestingly, after optic nerve crush, there was decreased neurite outgrowth and dendritic complexity, but no change in RGC soma size. However, in the microbead injection glaucoma model, the soma and dendritic parameters examined (including soma size, dendritic length, and dendritic complexity) showed increases 3 weeks after IOP elevation, which is the opposite of what was seen in the optic nerve crush model. By 6 weeks after IOP elevation, these differences were no longer statistically significant, perhaps due to residual presence of only those RGCs more resilient to damage.

In addition to the animal model used, additional caveats to analyzing RGC-type-specific dendritic alterations across multiple studies include different techniques for imaging RGCs and the time points used for analysis. Thus far we have discussed mouse models in which glaucoma can be induced in the eyes of transgenic animals in which RGCs are already sparsely labeled by GFP expression. The *DBA/2J* mouse strain is a spontaneous mutant with elevated IOP that has been studied quite extensively (Savinova et al. 1998). It was previously shown using this mouse model of experimental glaucoma that axonal atrophy, dendritic loss and remodeling, and cell soma shrinkage preceded ganglion cell death (Jakobs et al. 2005). Also of importance is the fact that these investigators did not observe any type specificity in RGC death, although this may be due to the late stages of examination when only sectors of spared RGCs remained. In order to examine dendritic architecture in more detail, Williams et al. quantified dendritic field area, total dendritic length, and performed Sholl analysis to examine complexity (Williams et al. 2013). When dendritic parameters were measured in *DBA/2J.Thy1-YFP* mice at different stages of disease, specifically mice which have not yet shown signs of cell loss or signs of early damage (NOE), mice with moderate damage (MOD), and mice with severe damage (SEV) assessed from axon counts, there was no significant difference in dendritic field area, total dendritic length, or Sholl plots among these groups. However, characteristic dendritic shrinkage was observed when RGCs were labeled by carbocyanine dyes, thereby raising the concern that *Thy1* may be downregulated in rodent models of glaucoma and subsequently result in downregulation of YFP expression, as reported by Huang et al. (2006). Therefore, a second approach to assess RGC dendrite morphology involved diolistically labeling the RGCs using the carbocyanine dyes DiI and DiO. In contrast to the findings using the *DBA/2J.Thy1-YFP* mice, the labeled RGCs showed a significant decrease in dendritic parameters between DBA/2J retinas with no or early damage (NOE) and controls. Interestingly, there was no significant difference in dendritic parameters between the MOD and SEV groups and controls. The authors postulate several explanations, including sample bias, regional bias in diolistic RGC labeling, bias of labeling only in remaining healthy neurons, or recovery of dendritic architecture. Given the

careful classification of the animal cohorts into NOE, MOD, and SEV groups based on optic nerve axon counts, this was the first study to definitively show that RGC dendritic shrinkage precedes significant axon degeneration.

To summarize, structural and functional changes of RGC dendrites appear to be influenced by the location and type of RGC (See Fig. 22.2). Feng et al. reported that a small subset of ON RGCs located in the dorsal retina of the *Thy1-YFP* mouse line are more likely to experience shrinkage of their dendritic arbor following IOP elevation (Feng et al. 2013). Recent progress in identification of RGC types more vulnerable to dendritic changes was made possible by the availability of transgenic mice labeling individual types of RGCs. El-Danaf and Huberman analyzed the dendritic complexity of multiple RGC types and discovered that RGCs with dendrites that laminate in the OFF sublamina display dendritic atrophy and rearrangements more readily than RGCs with dendrites that laminate in the ON sublamina (El-Danaf and Huberman 2015). Accordingly, multielectrode array recordings using the same mouse model of experimental glaucoma revealed that OFF-transient RGCs are the first type to experience functional receptive field size reduction (Della Santina et al. 2013). This "selective vulnerability" and differential involvement of RGC types in glaucoma may be critical in the early stages of the disease and thus important for the design of therapies before glaucoma reaches a late stage, when all types of RGCs are lost as observed in old DBA/2J mice (Jakobs et al. 2005).

22.4 Dendrite Remodeling in the Brain

While dendritic pruning in the retina appears to be a consistent feature across various animal models of experimental glaucoma, there is also evidence that dendritic atrophy is a hallmark of target neurons in the lateral geniculate nucleus (LGN) of the brain. Gupta et al. (2007) used MAP2 to label dendrites of LGN neurons in nonhuman primates with various degrees of optic nerve axon loss induced by argon laser scarification of the trabecular meshwork. Although the experimental design did not distinguish between LGN interneurons and relay neurons projecting to the visual cortex, both dendritic field area and dendritic complexity were significantly decreased in magnocellular layer 1 and parvocellular layer 6 compared to controls, even in cases where optic nerve axon loss was not detected. This study did not examine the retinas of these glaucomatous eyes, which would have been useful in determining the relationship between RGC dendritic degeneration in the retina with LGN dendritic atrophy. This group then went onto further detail the dendritic changes, specifically the LGN relay neurons that project to the primary visual cortex, using the same nonhuman primate model of glaucoma (Ly et al. 2011). Furthermore, they examined the effect of memantine, an NMDA glutamate open-channel blocker used as a neuroprotective agent to treat Alzheimer's disease, on dendrite morphologic parameters. As in the prior study, dendrite complexity and dendrite length were decreased in magnocellular layer 1 and parvocellular layer 6 compared to controls. In the memantine-treated group,

reduction of dendrite complexity and length was significantly less than in the vehicle-treated group, suggesting that targeting the LGN neurons in glaucoma may be a worthwhile treatment goal.

More recently, the temporal relationship between RGC degeneration in the retina and in the brain has been examined using a rat model of ocular hypertension (Liu et al. 2014). In this study, dendritic morphologic parameters were examined in RGCs, superior colliculus (SC), and LGN cells over time in the same rat model of chronic glaucoma. As early as 1 week after IOP elevation, significant RGC dendritic degeneration had occurred. In some severely damaged RGCs, only the cell bodies and primary dendrites could be identified. The authors separated the RGCs based on morphologic types (RI, RII, and RIII) and concluded that RGC loss was not selective for larger cells since after 1 week the smaller RII cells had the most damage. Dendritic alterations in the SC and LGN were also examined in detail (five types of SC cells and two types of LGN cells). All types of SC and LGN cells exhibited dendritic degeneration, with more severe and earlier damage in the SC compared to the LGN. One explanation for this result may be that the majority of retinal projections in the rodent project to the SC rather than LGN. This study was unique in its examination of the temporal relationship of dendritic changes among these cells in different locations along the visual pathway, and showed that RGC dendritic shrinkage occurred earliest, around 1 week after IOP elevation, followed by neurons in the SC and then the LGN around 4 weeks.

22.5 Changes in Morphology vs. Function

The majority of functional data evaluating RGC status in glaucoma comes from mass recording of retinal activity, such as the pattern electroretinogram (PERG) (Porciatti 2015). Both in animal models and humans, this technique can be used to monitor disease progression reflected by progressively lower responses that parallel RGC death. Unfortunately, this approach does not provide information about function at the single-cell level, which is required for correlating changes in RGC morphology with alterations in function.

What is the relationship between the structural changes in dendrites and synapses and the functional changes observed in RGCs upon IOP elevation in experimental glaucoma models? Figure 22.3 delineates our current thinking with regard to the timing of morphologic and physiologic alterations that occur to RGCs in glaucoma. In early degeneration, one of the earliest morphologic changes is the loss of excitatory synapses, which precedes any structural changes to the dendrites (Fig. 22.3, second column). Functionally, there is decreased spontaneous firing of RGCs along with decreases in light responses and sensitivity. However, there is no change in receptive field size. Reduction of light sensitivity as well as cation currents for ON-sustained RGCs and chloride currents for OFF-sustained RGCs occur without alteration of bipolar cell light responses in multiple mouse models of experimental glaucoma (Pang et al. 2015). This recent evidence suggests that the

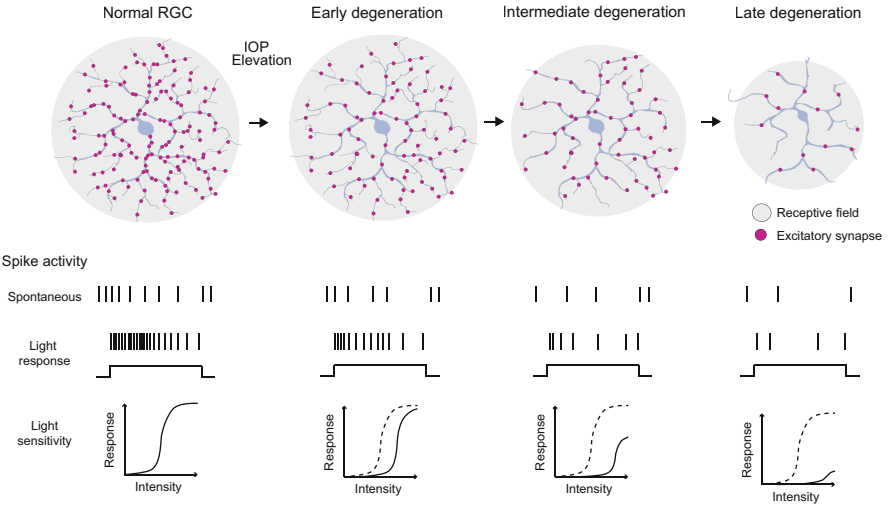


Fig. 22.3 Proposed sequence of morphologic and functional changes during RGC degeneration in experimental glaucoma. Early on in degeneration, excitatory synapses are eliminated, representing the first structural change of the retinal ganglion cell (RGC) in response to intraocular pressure (IOP) elevation. Coincident with the loss of synapses is diminished spontaneous activity and light responses. In intermediate stages of degeneration, the dendritic arbor shrinks, with further loss of function, most notably a decrease in the size of the functional receptive field. Finally, at late stages of degeneration, marked dendritic atrophy occurs, the cell soma shrinks, and the RGC eventually dies. Light sensitivity (*bottom row*) of degenerating RGCs (*solid line*) is progressively lower compared to control RGCs (*dashed line*). Similarly, maximal response amplitude decreases in parallel with the loss of excitatory synapses. The proposed sequence depicted here is summarized from Della Santina et al. (2013)

early impairment in RGC response sensitivity arises from reduced transmission between presynaptic partners and RGCs. Rod pathway-driven responses are reduced primarily due to a lack of input onto AII amacrine cells (Pang et al. 2015). Cone bipolar-driven responses may be altered due to a reduced number of excitatory synapses onto RGCs, as observed for ON- and OFF-sustained alpha RGCs early after IOP elevation, when their dendritic arbor morphology is unaltered (Della Santina et al. 2013).

At the intermediate stage of degeneration, there is further excitatory synapse loss, and the dendritic arbor begins to shrink, including decreased total dendritic length and dendritic complexity (Fig. 22.3, third column). Spontaneous activity and light responses further diminish. Indeed, Weber and Harman (Weber and Harman 2005) reported decreased responses in parasol RGCs to both spatial and temporal stimuli, in a nonhuman primate model of glaucoma where the same cells show dendritic arbor shrinkage. Although less responsive after glaucoma induction, parasol cells preserve normal phasic response to electrical stimulation (Weber and Harman 2005). Reduced response sensitivity has also been observed for alpha-like ganglion cells in the mouse by patch-clamp recording of their light responses (Pang et al. 2015).

Most notably, at this stage of degeneration, the functional receptive field has also shrunk. In the microbead injection mouse model, the reduction of dendritic arbor size coincides with a decrease in the size of the RGC's functional receptive field, a property that is evaluated by multielectrode array (MEA) recordings of RGC light responses (Feng et al. 2013; Della Santina et al. 2013).

Finally, at late stages of degeneration (Fig. 22.3, last column), there is considerable morphologic and functional loss of the RGC, including loss of entire dendritic branches and cell body shrinkage, along with loss of the light response and, finally, activation of apoptosis. While this is an overall framework of the morphologic and functional events in glaucomatous neurodegeneration, it remains to be determined how all of the different RGC types respond differentially to IOP elevation, both in terms of the tempo of perturbations and their specific characteristics.

While dendritic and synaptic changes impair RGC functionality, possibly up to the point where cell death mechanisms are initiated, a few recent reports suggest that mechanisms compensating for dendritic and synaptic degeneration may be engaged by RGCs. Interestingly, El-Danaf and Huberman found that for an individual ON-OFF RGC, dendrites stratifying in the OFF sublamina of the IPL degenerate, whereas dendrites in the ON sublamina expand their length and complexity (El-Danaf and Huberman 2015). This finding raises the possibility that a homeostatic mechanism in the RGC is evoked in response to the loss of OFF dendrites, possibly in an attempt by the cell to increase synaptic connectivity of the remaining dendrites and thus retain as much input as possible. Furthermore, Park et al. found that although there is a reduction in the total number of excitatory ribbon synapses in the IPL after IOP elevation, pre- and postsynaptic protein expression and the number of immature ribbon synapses were increased, suggesting that loss of dendrites may actually trigger synaptogenesis (Park et al. 2014).

22.6 Summary and Future Directions

Despite the increasing knowledge about dendritic degeneration in glaucoma, the cellular mechanisms underlying dendritic pruning and synapse loss are not yet well understood. It is becoming increasingly clear that not all RGCs degenerate with the same time course following ocular hypertension. In particular, OFF RGCs are reportedly more readily subjected to functional and morphologic alterations, whereas ON RGCs are more resilient. However, we recognize that until a complete RGC atlas and ability to identify individual types of RGCs is achieved, we will not fully understand which and why some RGC types are more vulnerable and others more resistant to damage in glaucoma. The cellular mechanisms driving this "selective vulnerability" remain to be elucidated. In parallel, a more in-depth characterization of the degeneration of each type of RGC will help reveal what RGC functional properties are rapidly perturbed and could be detected as specific vision defects.

Mechanisms driving the rearrangement of dendrites and synapses in glaucoma models are poorly understood. In vivo imaging of the degeneration process in action longitudinally should offer more detailed insight into how glaucoma leads to remodeling of synapses and dendritic architecture of the RGCs. Monitoring synaptic activity in various regions of the RGC dendritic tree could potentially reveal whether synapses that are preferentially eliminated belong to a particular input type, region of the dendritic tree, or display a characteristic level of activity. Unveiling the dynamic changes in degenerating RGC dendrites could also be an important step not only for identifying the initial effects of degeneration, but potentially also to reveal the critical steps needed for dendritic regeneration. Progress is already being made in the area of axonal regeneration, with certain RGC types demonstrated to have greater regenerative capacity (Duan et al. 2015).

From a therapy standpoint, it would also be of interest to identify compensatory mechanisms put in place by RGCs that are most resilient to degeneration. Such knowledge may provide key information to aid the design of strategies to slow down the disease. Therapeutic approaches will need to take into account the differential responses of RGC types to the insult, as well as capitalize on the differential timing of the steps leading to cell death in distinct RGC populations. Indeed, it will be necessary to tailor the intervention according to the disease state. Effective therapies administered at early stages of the disease could be aimed at preventing further RGC dysfunction and death, whereas late-stage interventions would require the repair of massively disrupted neural networks.

Dysfunction and death of neurons in both the outer and inner retina has been reported in experimental glaucoma models (Hernandez et al. 2009; Fernández-Sánchez et al. 2014), although these changes occur at later time points compared to damage and dysfunction of RGCs (Agudo-Barriuso et al. 2013). An in-depth analysis of the early changes in those neurons will reveal whether bipolar cells and amacrine cells that contact RGCs exhibit any compensatory behavior or remodel their connectivity over time. Moreover, current research and screening methods are particularly focused on the impact of degeneration on excitatory neurons, whereas alterations to inhibitory neurons remain unknown. Rearrangement in amacrine cell connections would suggest that inhibitory connections in the retina are also modified. Furthermore, changes in the outer retina involving photoreceptor synapses also need to be documented more thoroughly. Only when a comprehensive examination of how degeneration of the RGC, the last station of retinal processing, affects the entire retinal circuit will we attain a more complete understanding of how the CNS reacts and rearranges in the context of degenerative diseases in which the insult appears targeted to a single class of neuron.

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