



Critical pathogenic events underlying progression of neurodegeneration in glaucoma

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ABSTRACT

Glaucoma is a common optic neuropathy with a complex etiology often linked to sensitivity to intraocular pressure. Though the precise mechanisms that mediate or transduce this sensitivity are not clear, the axon of the retinal ganglion cell appears to be vulnerable to disease-relevant stressors early in progression. One reason may be because the axon is generally thin for both its unmyelinated and myelinated segment and much longer than the thicker unmyelinated axons of other excitatory retinal neurons. This difference may predispose the axon to metabolic and oxidative injury, especially at distal sites where pre-synaptic terminals form connections in the brain. This idea is consistent with observations of early loss of anterograde transport at central targets and other signs of distal axonopathy that accompany physiological indicators of progression. Outright degeneration of the optic projection ensues after a critical period and, at least in animal models, is highly sensitive to cumulative exposure to elevated pressure in the eye. Stress emanating from the optic nerve head can induce not only distal axonopathy with aspects of dying back neuropathy, but also Wallerian degeneration of the optic nerve and tract and a proximal program involving synaptic and dendritic pruning in the retina. Balance between progressive and acute mechanisms likely varies with the level of stress placed on the unmyelinated axon as it traverses the nerve head, with more acute insult pushing the system toward quicker disassembly. A constellation of signaling factors likely contribute to the transduction of stress to the axon, so that degenerative events along the length of the optic projection progress in retinotopic fashion. This pattern leads to well-defined sectors of functional depletion, even at distal-most sites in the pathway. While ganglion cell somatic drop-out is later in progression, some evidence suggests that synaptic and dendritic pruning in the retina may be a more dynamic process. Structural persistence both in the retina and in central projection sites offers the possibility that intrinsic self-repair pathways counter pathogenic mechanisms to delay as long as possible outright loss of tissue.

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1. The neuroscience of glaucoma

1.1. Glaucoma and ocular pressure

Glaucoma is an etiologically complex collection of optic neuropathies. For the most prevalent primary forms, stressors related to age and intraocular pressure (IOP) lead to progressive degeneration of the retinal projection to the brain (Calkins and Horner, 2012; Nickells et al., 2012). This definition has evolved from many traditional viewpoints in two important ways. In terms of etiology, rather than linking exclusively to elevated IOP (ocular hypertension) for primary glaucoma, which remains a prominent risk factor, the definition generalizes to include IOP of any magnitude including the nominally normal range. IOP-related stress generally, but not always, takes place on a backdrop of other age-related stressors that influence susceptibility for vision loss (Burgoyne, 2011). Indeed disease incidence increases with age even when accounting for elevated IOP (Leske et al., 2007). In terms of effects of the disease, rather than focusing on the apoptotic death of retinal ganglion cells (RGCs), whose axons comprise the optic projection to the brain (Fig. 1), the definition emphasizes degenerative events along the entire optic projection (Nickells et al., 2012; Howell et al., 2012a; Levin, 1999). In doing so, the tacit implication is that events distal to the RGC cell body are fundamental for understanding both the progression of neurodegeneration and ultimate vision loss (Whitmore et al., 2005). The argument is made that RGC apoptosis, while doubtlessly relevant in progression, may not be the most meaningful feature of pathogenesis in terms of seeking novel interventional targets (Chang and Goldberg, 2012; Nickells, 2007).

Glaucoma is typically (but not exclusively) associated with IOP, which remains the only treatable risk factor and therefore the predominant independent variable in animal models (McKinnon et al., 2009; Morrison et al., 2011; Pang and Clark, 2007; Sappington et al., 2010). Glaucoma's association with IOP is a defining, yet complex feature of the disease. A substantial fraction of patients never present elevated IOP at all, but nevertheless demonstrate clinical progression similar to those who do (Heijl

et al., 2002; Iester et al., 2012; Shields, 2008). Some with ocular hypertension never proceed to glaucomatous vision loss. From a clinical standpoint, many patients continue to progress despite regimens to manage IOP (Heijl et al., 2011; Pascale et al., 2012). For them, there is no clinical practice to prevent further loss of vision or to restore lost function. Once pathogenesis has proceeded past a critical point, irreversible loss of function is (for now) inevitable. Thus, emerging views of glaucoma with a nod toward clinical translation tend to deemphasize the apoptotic loss of RGCs, which we know now occurs later in animal models of progression, and focus more on the axonal projection, which is affected quite early.

1.2. Glaucoma and the central nervous system

Neuroscience-based approaches to understanding pathogenesis in glaucoma have surged, not only from the standpoint of preserving vision, but also because studies of pathogenesis in glaucoma have evolved as tools to inform and interrogate other degenerative disorders of the central nervous system (CNS). This surge brings with it the hope of better understanding the mechanisms of glaucomatous neurodegeneration and of identifying neuroprotective therapies independent of IOP management. The broader view expects that some of these may translate to interventions for other diseases, beginning with other optic neuropathies. For example, the potent and highly selective α_2 -adrenergic agonist brimonidine tartrate has direct neuroprotective efficacy for a variety of insults in animal models (Saylor et al., 2009), including axonopathy induced by elevated IOP (Lambert et al., 2011).

Even so, we must caution against any tendency to view glaucoma as a dichotomy: as either a disease of the eye involving ocular hypertension or as one of the CNS in cases where elevations in IOP are absent. Emphatically, this is an unnecessary and misleading distinction. It is a misinterpretation of the disease to conclude that glaucoma in the absence of ocular hypertension *taken as a whole* must evolve from a separate etiology rooted in the CNS and independent of IOP altogether. Certainly other stressors, some systemic, are known to induce glaucomatous neurodegeneration without elevated IOP (Wax et al., 2008). Etiologically, these may contribute

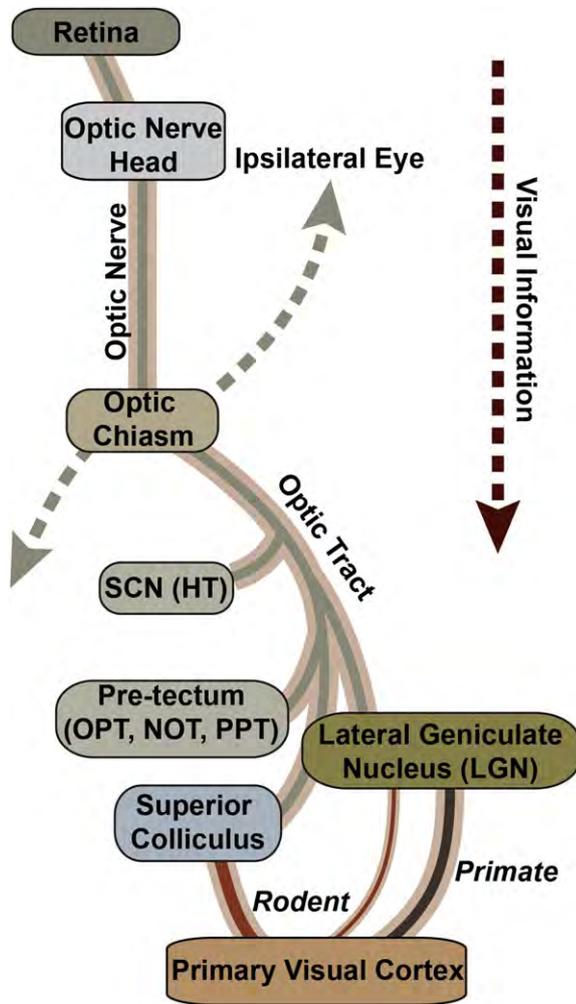


Fig. 1. Retinal projection in the mammalian brain. Schematic of contralateral optic projection, which dominates in rodents. The axons of retinal ganglion cells (RGCs) exit the retina through the optic nerve head to form the nerve proper. The nerves from the two eyes meet at the optic chiasm, which parses axons to either the ipsilateral or contralateral optic tract in the brain. Central targets for RGC axons include the suprachiasmatic nucleus (SCN) of the hypothalamus (HT) and several subcortical midbrain nuclei lying distal to it. These include the olivary pretectal nucleus (OPN), the nucleus of the optic tract (NOT), and the posterior pretectal (PPT) nucleus. In primates, the lateral geniculate nucleus (LGN) of the thalamus is the dominant RGC relay to the primary visual cortex, while in rodents the more distal superior colliculus of the midbrain dominates with a smaller LGN projection to the cortex.

to degeneration in a subset of those patients considered to have normal tension glaucoma. But just as glaucoma spans a broad IOP range, so too do those patients who respond with improved outcome to IOP-lowering therapies, including many with normal IOP (Leske et al., 2003). Thus, the absence of ocular hypertension does not translate *a priori* to categorical independence from an IOP-based etiology. The strongest hypothesis that we can pose is that glaucoma is not about *elevated* IOP but rather *sensitivity* to IOP itself.

This hypothesis has enormous ramifications for how we approach neurodegeneration in the disease. We must consider states of the neural system in which IOP-related stressors could be injurious. Such states must be analyzed in the context of mechanisms that could mediate or transduce sensitivity to pressure. This also involves understanding how variability within these mechanisms might underlie individual susceptibility to injury, i.e., why IOP is stressful for some eyes but not for others (Burgoyne, 2011). It is also necessary to understand how neurodegeneration progresses

from the transduction of IOP-related stress to vision loss in the disease. This understanding is predicated upon careful dissection of neuronal, glial and vascular mechanisms that promote progression (Almasieh et al., 2012). Finally, we must challenge ourselves to avoid a unidirectional viewpoint of glaucoma and consider how the neural system might respond to IOP-related stress to minimize injury or isolate damage already incurred. This response is of particular importance for identifying intrinsic factors that could be exploited and developed as novel neuroprotective therapies independent of IOP management.

2. Primary RGC vulnerability in glaucoma

2.1. The RGC circuit

The human retina contains roughly 1.5 million RGCs distributed among several types defined by a unique combination of morphologically, neurochemical and physiological parameters (Hendry and Calkins, 1998). Specialized tuning of each RGC type emerges from the complex summation of signals derived from distinct presynaptic circuits. The canonical feed-forward circuit in the retina is comprised of three classes of excitatory, glutamatergic neurons: photoreceptors, bipolar cells, and of course RGCs. Synaptic transmission occurs at two levels of connectivity: photoreceptor → bipolar cell in the outer retina and bipolar cell → RGC in the inner retina (Fig. 2). Excitatory signaling at both levels is modulated by two major classes of interneurons: horizontal cells in the outer retina, which are GABAergic, and amacrine cells in the inner retina, which are mostly (but not entirely) GABAergic and glycinergic (Oesch et al., 2011; Wassle and Boycott, 1991). Three major classes of glia (astrocytes, microglia and Müller cells) in the retina contribute both to the homeostatic environment of the RGC and to its response to disease-relevant stressors through a variety of signaling cascades (Johnson and Morrison, 2009; Tezel, 2008).

2.2. RGCs as direct targets in glaucoma

Like all optic neuropathies, the defining pathophysiological event in glaucoma is *primary* degeneration of the RGC and its axonal projection. The term “primary” is used in this context to mean that pathogenic mechanisms work against normal function of the RGC *directly*, though other cells and structures may suffer *indirectly* or *secondarily* to RGC degeneration. This is an important distinction, and one that should not be confused with indirect degeneration of additional RGCs secondary to a focal lesion, as in partial nerve transection (Levkovitch-Verbin et al., 2001). It is often assumed that primary injury to RGCs translates to *selective* injury, and many testimonials begin exactly with that definition of the disease. This assumption underscores the importance of asking whether other cells of the retina or optic projection are also susceptible as primary targets in parallel with RGCs or, what we presume would be more likely, undergo secondary degeneration once RGC pathogenesis begins. This subject has been equivocal and, at times, highly controversial (Quigley, 2001). As might be expected, studies of either human patients using imaging approaches or of human tissue post-mortem are accented by a large degree of unpredictability due to differences in progression, age and type of glaucoma. Thus, the variability between studies is immense (e.g., (Kendell et al., 1995) vs. (Fan et al., 2011)), as has been the range of conclusions from them.

2.3. Susceptibility of other retinal neurons

2.3.1. Photoreceptors

A particular emphasis has been placed on photoreceptor survival in glaucoma, since this factor could most obviously

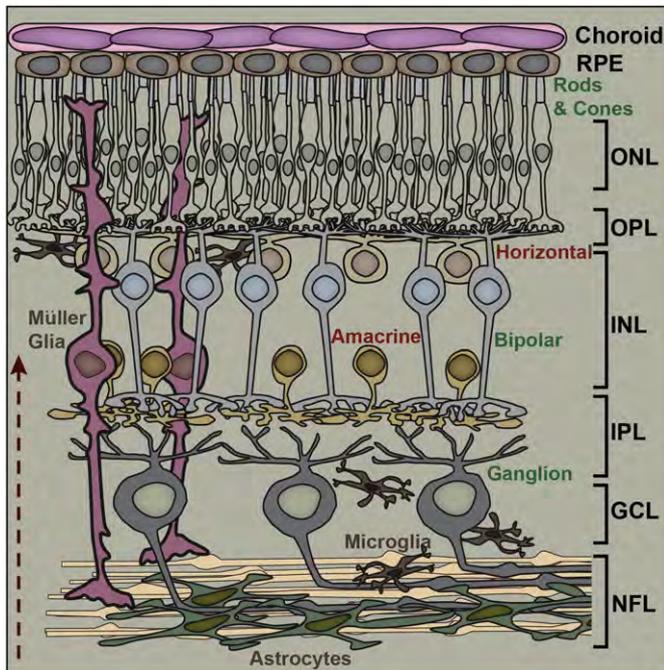


Fig. 2. Fundamental retinal circuitry. Basic retinal circuit includes three classes of excitatory (glutamatergic) neurons: rod and cone photoreceptors, bipolar cells, and retinal ganglion cells (RGCs). Synaptic transmission between photoreceptors and bipolar cells is modulated by inhibitory (GABAergic) horizontal cells in the outer plexiform layer (OPL). Amacrine cells (mostly GABAergic and glycinergic) modulate transmission between bipolar cells and RGCs in the inner plexiform layer (IPL). Müller glia have cell bodies in the inner nuclear layer (INL) along with bipolar, amacrine and horizontal cells and extend processes radially throughout the retina. Astrocyte glia form a dense plexus over RGC axons in the nerve fiber layer (NFL), while microglia distribute broadly. Arrow indicates the path of light. Other abbreviations: RPE (retinal pigment epithelium), ONL (outer nuclear layer), and GCL (ganglion cell layer).

influence the interpretation of visual field measurements in patients. An early histological study of human donor tissue found no association between photoreceptor number and disease severity in glaucomatous patients (Kendell et al., 1995), which was mirrored by results in a non-human primate model (Wyganski et al., 1995). Another monkey study demonstrated decreased expression of cone opsin mRNA in the mid-peripheral retina; similar changes occurred in human glaucomatous retina at the same location, but without confirmed photoreceptor loss (Pelzel et al., 2006). However, a more recent analysis described swelling and patchy loss of cone photoreceptors in the macular region of some human glaucomatous retina and similar swelling of cones (but without loss) in monkeys with laser-induced hypertension (Nork et al., 2000). Increased thickness of the foveal photoreceptor layer in mildly glaucomatous human retina is also consistent with swelling (Fan et al., 2011).

Photoreceptor pathology appears to be spatially correlated with damage to the inner retina. A recent multi-modal imaging study of 10 patients across various ages and glaucoma subtypes found patchy compromise of cone photoreceptor density at retinal locations where visual field sensitivity and inner retinal structure also reflected damage (Choi et al., 2011). Similarly, a careful comparison of neuronal cell body populations in human glaucomatous retina found that compared to age-matched controls, loss of RGCs in the perifoveal region was spatially correlated with thinning of both the inner and outer nuclear layers (Lei et al., 2008). Loss of RGCs in the region of the retina quantified was about 45%, roughly four-fold greater than either the loss of inner nuclear cell bodies or of photoreceptors in the outer retina (Lei et al., 2008). Incidentally, the

same approach (applied by the same group) found in aged human retina that thinning of the RGC population is spatially-correlated with modest loss of inner nuclear layer neurons and more extensive loss of photoreceptors (Lei et al., 2011). In that study, RGC loss over the lifetime was about 25% and photoreceptor loss about 15% compared to young retina (Lei et al., 2011); see also (Curcio and Allen, 1990). Taken as a whole, these studies indicate that RGC degeneration is accompanied at least by morphological indications of photoreceptor pathology that, as pathogenesis progresses, is likely to result in outright loss of cells. This conclusion is supported by a number of physiological studies of outer retina dysfunction in human patients (Vaegan et al., 1995; Velten et al., 2001) and in inducible monkey, mouse and rat models (Cuenca et al., 2010; Mittag et al., 2000; Raz et al., 2003).

The DBA (“dilute brown non-agouti”) mouse is one of the oldest known inbred strains and perhaps the best-characterized chronic model of glaucoma, presenting age-dependent (but variable) elevations in IOP due to iris atrophy and pigment dispersion (Anderson et al., 2002; Chang et al., 1999; Howell et al., 2007b; John et al., 1998). Early studies of the DBA did not find photoreceptor or other non-RGC neuronal loss, the implication being that pathology in the retina was RGC-specific (Jakobs et al., 2005; John et al., 1998; Libby et al., 2005a; Schuettauf et al., 2004). However, a comparison of electroretinogram (ERG) measurements with retinal histology reported attenuation of scotopic a- and b-wave amplitude concurrent with thinning of the outer retina and of the inner synaptic layer, respectively (Bayer et al., 2001). This reduction in a- and b-wave response has been corroborated more recently by independent groups (Harazny et al., 2009; Heiduschka et al., 2010), and stands in contrast to the photopic flash ERG, which remains largely intact in the DBA until late in progression (Saleh et al., 2007).

2.3.2. Retinal interneurons

Other cells in the DBA inner retina may also be susceptible. For retinas in which about half of the RGCs were lost compared to age-matched C57 mice, GABAergic types of amacrine cell were reduced by roughly the same fraction; the cholinergic subtype was particularly vulnerable (Moon et al., 2005). These results cannot be explained simply by lower starting numbers of neurons in the DBA retina. The young DBA retina has comparable numbers of cell bodies in each of the three nuclear layers compared to C57; these diminish with progression (Bayer et al., 2001). Interestingly, in older DBA retina, as RGCs degenerate, both glycinergic amacrine cells and horizontal cells (GABAergic) remain comparable in number to the C57 retina (Moon et al., 2005). Similarly, in a rat glaucoma model, even as RGCs diminish in number both GABAergic and glycinergic amacrine cells remain intact; this is not so with nerve transection (Kielczewski et al., 2005). While glial hypertrophy is an early hallmark of progression in the DBA retina, the numbers of Müller glia do not change (Inman and Horner, 2007). This does not preclude their contributing physiologically to changes in RGC function early on (Saleh et al., 2007).

2.3.3. Mechanism of secondary degeneration

Based on the available literature, it is reasonable to conclude that during progression other retinal neurons besides RGCs also may be vulnerable, at least eventually. However, there is little to suggest they represent primary targets independent of RGC degeneration. Importantly, glaucomatous pathology in other retinal layers appears to be spatially correlated with and depends upon the extent of RGC degeneration (Choi et al., 2011; Lei et al., 2008). This correlation is the best evidence that secondary degeneration is trans-neuronal from the RGC and does not simply represent diffuse, residual damage. In the most carefully quantified human

glaucomatous retina, there was a gradient of correlated cell loss from RGCs to the inner nuclear layer to the outer nuclear layer (Lei et al., 2008). This stands in contrast to the normal aged retina in which significant RGC and photoreceptor loss sandwich a less severe (but still spatially correlated) reduction in cells of the inner nuclear layer (Lei et al., 2011). This pattern suggests that age-related loss of RGCs and photoreceptors are dependent not upon one another, but rather conjointly upon a spatially-specific pathway that influences both sets of neuron.

Given this correlation, it is tempting to envision a degenerative cascade in glaucoma in which substantial reduction in RGCs leads to eventual trans-synaptic loss of bipolar cells and finally photoreceptors in retrograde progression. There is precedence for this kind of secondary degeneration elsewhere in the RGC pathway. For example, ablation of the primary visual cortex (V1) in non-human primates induces apoptotic degeneration of cortical-projecting neurons in the lateral geniculate nucleus (LGN) followed by retrograde degeneration of the optic projection and of RGCs sometime later (Cowey et al., 2011; Johnson and Cowey, 2000; Mihailovic et al., 1971; Niida et al., 1990; Van Buren, 1963). A similar pattern of degeneration occurs in human hemianope patients, albeit with greater individual variability, and does so whether the condition is congenital or due to traumatic injury (Cowey et al., 2011; Jindahra et al., 2009). These examples illustrate how an anatomically distal stressor (e.g., a cortical lesion) can affect survival of more proximal neurons (e.g., RGCs) in retrograde order. For glaucoma and its experimental models, we presume that RGC injury represents the distal stressor that affects retrogradely the survival of upstream retinal neurons.

However, the analogy with cortical injury only goes so far. First, in terms of thinning of the inner nuclear layer, there is little evidence to support the idea that bipolar cells are more susceptible than either amacrine neurons or Müller glia. In fact, in the DBA retina two of the most common bipolar cell types are unaffected during progression, with the available data suggesting far greater loss of GABAergic amacrine cells (Moon et al., 2005), which are presynaptic to both RGCs and bipolar cells (Calkins and Sterling, 1996). In direct comparisons, RGC degeneration is *always* overwhelmingly greater in amplitude and more wide-spread retinotopically. For example, in aged DBA retina with few or no remaining RGCs and very little residual visually-evoked potential in the brain, a- and b-wave ERG signals were reduced by only 40% compared to C57 mice (Heiduschka et al., 2010). In the study of human tissue by Nork et al. (2000), of those retinas with severe RGC dropout (>90%), photoreceptor pathology was observed in only 25% of the retina, with 35% having no loss.

2.4. Why are RGCs primarily susceptible in glaucoma?

In the retina, glutamatergic signaling from photoreceptor to bipolar neuron to RGC is accomplished without myelination of axons. What distinguishes the RGC axon is that it exits the eye and penetrates the laminar region of the nerve head where, in doing so, it *becomes* myelinated and continues through the nerve proper on its way to central targets in the brain (Fig. 3). Thus, it has been appreciated for decades that RGC susceptibility in glaucoma must be at least in part axogenic – derived from the properties of the axon and its local milieu in the nerve (Levin, 1999). Indeed some of the earliest mechanistic work addressing RGC degeneration focused on axogenic mechanisms, particularly due to extrinsic factors at the optic nerve head. This is covered amply in earlier reviews (Burgoyne et al., 2005; Hernandez, 2000; Osborne et al., 2001; Quigley, 1999). It is worth considering whether factors intrinsic to the RGC axon itself could render it particularly vulnerable to insult.

2.4.1. Most RGC axons are thin

Axons are particularly metabolically demanding and require a consistent source of adenosine-5'-triphosphate (ATP) available for hydrolyzation. This source must be sufficient to maintain anterograde transport from the retina to the brain, retrograde transport from the brain to retina, and saltatory conduction between the cell body and axonal terminals (Carelli et al., 2004; Hollenbeck, 1996). Physiological studies of parallel visual pathways to the LGN in primates highlight the faster axonal conduction velocity of large, magnocellular-projecting RGCs (mostly parasol cells) compared to that of the smaller and more numerous parvocellular-projecting midget-type RGCs (Callaway, 2005; Wassle and Boycott, 1991). Given the difference in conduction velocities, it is tempting to conceive of a bimodal distribution of small and large axon diameters. In point of fact, the distribution of axon sizes in the optic nerve is continuous, very narrow and heavily skewed toward small axons, as it is in other white-matter tracts (Reeves et al., 2012; Wang et al., 2008a). Most RGC axons are 0.2–0.7 μm in diameter; this is so from primates to rodents despite large differences in total length of the optic nerve (Perge et al., 2009).

While the largest (and rarest) RGC axons are 1.5–2.0 μm in diameter, auditory axons range from 1 to 4 μm and vestibular axons from 0.2 to 9 μm (Perge et al., 2012). The relatively small size of RGC axons appears to be optimized for both information rate (action potential production) and energy consumption, so that firing rate is set to the minimal level to maintain communication with central targets (Niven and Laughlin, 2008; Perge et al., 2009, 2012; Wang et al., 2008a). In other CNS regions, there is also a large difference in axon diameter between unmyelinated and myelinated axons even when the thickness of the myelin sheath is taken into account (Reeves et al., 2012; Wang et al., 2008a). Importantly, this is not true of the retina and optic nerve, where the distributions of RGC axon diameters are very similar (Perge et al., 2009, 2012), with continuity in their inner diameter as they pass through the laminar region of the nerve head (Minckler et al., 1976; Yu Wai Man et al., 2005). This could have important implications for glaucoma, as discussed below.

2.4.2. RGC axons and mitochondria

One purpose of myelination is to increase axonal efficiency by reducing energy consumption. Unmyelinated axons, in contrast, are relatively inefficient, requiring far greater energy to maintain axonal conduction owing to the absence of saltatory conduction (Perge et al., 2009; Waxman, 1978). This is reflected in the greater abundance of mitochondria in the unmyelinated portion of the RGC axon, creating a sharp gradient between the two compartments (Bristow et al., 2002; Morgan, 2004; Yu Wai Man et al., 2005). In the nerve fiber layer of the retina, RGC axons contain intermittent varicosities that are packed with mitochondria and mark points of inter-axonal and axonal-astrocyte contact (Wang et al., 2003). Most of the mitochondria in this region are contained in RGCs and their axons, unlike the optic nerve where an estimated 70% of the mitochondria are in astrocytes (Perge et al., 2009). In terms of energy usage, the unmyelinated axon is roughly 10-fold less efficient than the myelinated axon in conveying an action potential for the most common of axon sizes (Perge et al., 2009). This segment of the nerve head also has a higher density of voltage-gated Na^+ channels, reflecting the higher energy demand for conduction (Barron et al., 2004).

Mitochondria are trafficked in both anterograde and retrograde directions along microtubule and actin molecular tracks in response to physiological signals (Hollenbeck, 1996; Hollenbeck and Saxton, 2005). While these signals are not entirely understood, they include local ATP demand and increased Ca^{2+} influx, which is countered in part by mitochondrial buffering (Hollenbeck

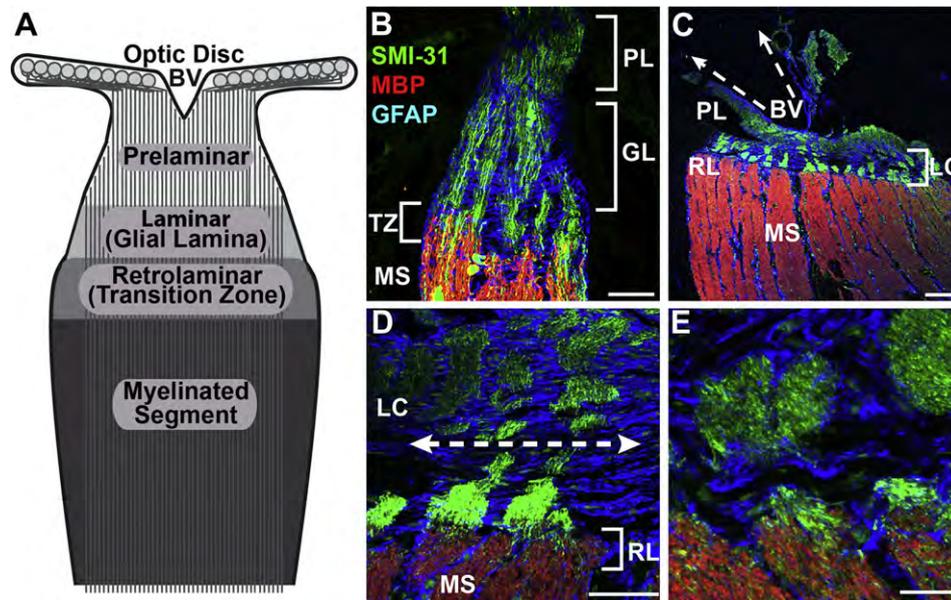


Fig. 3. Structure of the optic nerve head. A: Primary zones of the mammalian optic nerve head. Optic disc marks exit of unmyelinated RGC axons from the retina to the prelaminar zone of the nerve head and the conduit for blood vessels (BV). The laminar region (glial lamina in rodents) contains a concentration of astrocytes that separate bundles of axons. The retrolaminar zone marks the transition to oligodendrocyte-derived myelination of axons. B: Immuno-fluorescent confocal micrograph through C57 mouse nerve head labeled for phosphorylated neurofilaments (SMI31) in axons, glial fibrillary acidic protein (GFAP) for astrocytes, and myelin basic protein (MBP). Prelaminar region (PL), glial lamina (GL), transition zone (TZ) and myelinated segment of nerve are indicated. C: optic nerve of squirrel monkey shows well-defined lamina cribrosa (LC) and retrolaminar (RL) region. D: higher magnification of monkey nerve head demonstrates lateral tiling of GFAP-labeled astrocyte processes and fascicles of RGC axons. E: higher magnification of retrolaminar zone in monkey shows transition to myelination. Scale = 50 μm (B, D); 100 μm (C); 20 μm (E).

and Saxton, 2005; Nicholls and Budd, 2000; Rintoul and Reynolds, 2010). Any condition that inhibits mitochondrial motility, such as diminished kinesin-mediated anterograde transport, can lead to the accumulation of toxic free radicals, amplified mitochondrial permeability, increased cytochrome c and oxidative stress (Lee et al., 2010; Rintoul and Reynolds, 2010; Wallace, 1999). Many of these elements are in fact observed in various models of glaucoma (Almasieh et al., 2012). In the retrograde direction, diminished motility can prevent the retrieval of dysfunctional mitochondria to the cell body for autophagic degradation (Rintoul et al., 2003). Early observations of the sharp gradient in mitochondrial concentration between the unmyelinated pre-laminar segment and the myelinated post-laminar region were construed as constricted motility and accumulation, rather than reflective of the intrinsic energy demand (Hollander et al., 1995; Minckler et al., 1976). These conclusions are likely unwarranted (Barron et al., 2004; Morgan, 2004), and any consideration of mitochondrial accumulation at the transition zone must consider the normal gradient there (Munemasa et al., 2010; Yu Wai Man et al., 2005).

A variety of intracellular conditions can lead to diminished mitochondrial transport within axons (Rintoul and Reynolds, 2010), including an overload of glutamate that is associated with a transformation of mitochondria from an elongated morphology to round (Rintoul et al., 2003). Yu Wai Man et al. (2005) have argued that such conditions could underlie a variety of mitochondrial-related hereditary optic neuropathies. In terms of the RGC, one can imagine scenarios in which either increased ATP demand along the distal axon or accumulation of stressed mitochondria in the cell body or unmyelinated axon would require rapid transport from the region of high mitochondrial concentration (unmyelinated segment) to the low (myelinated segment). Alternatively, disease states are often associated with increased mitochondrial volume of many orders of magnitude, which can greatly reduce motility (Chang and Reynolds, 2006). In either case, the RGC axon is at a disadvantage without the benefit of enlargement of the axon at

the transition between unmyelinated to myelinated segment to minimize a bottleneck.

A uniformly small axon is also at a disadvantage should available ATP diminish rapidly, namely at distal sites along the myelinated axon and pre-synaptic active zones in central targets. An obvious consequence of reduced ATP is diminished capacity for action potential signaling along the axon due to inhibition of Na^+/K^+ ion pumps (Ames, 2000). However, failure of the Na^+/K^+ ion exchange also induces accumulation of axoplasmic Na^+ which in turn promotes reverse operation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger leading to intra-axonal Ca^{2+} accumulation (Stys, 2004). Because axons are cylindrical, a smaller inner diameter translates to a higher surface-to-volume ratio. This implies less available cytoplasmic volume to buffer each local influx of Ca^{2+} (Reeves et al., 2012; Stys, 2004). We have reviewed elsewhere how excessive cytosolic Ca^{2+} activates Ca^{2+} -dependent enzymes such as calpains and phospholipases that can induce irreversible injury by degradation of axonal cytoskeleton (Crish and Calkins, 2011). More generally, small axons are preferentially vulnerable in many conditions, particularly those in which anoxia is a factor (Bechtold et al., 2005).

The unmyelinated axons of the photoreceptor and bipolar cell, unlike the RGC axon, are fairly short. The longest of the photoreceptor axons serving foveal cones are 200–400 μm in length with very few mitochondria (Hsu et al., 1998). Most of the mitochondria are in the inner segment, reflecting a higher metabolic demand than the rest of the photoreceptor (Perge et al., 2012). Furthermore, though the unmyelinated RGC axon in the retina can be 50-fold longer than the cone axon, its mean diameter is half as thick (Perge et al., 2012). For unmyelinated axons, the consequences of a small inner diameter are even more devastating. The membrane-to-axoplasm ratio is even higher, which promotes a robust differential between available sites for Ca^{2+} influx and available cytosolic volume to buffer Ca^{2+} accumulation (Stys, 2004). This difference may underlie increased vulnerability of thin unmyelinated fibers in both traumatic injury and Parkinson's disease (Braak et al., 2004;

Reeves et al., 2007, 2012). The vulnerability of the RGC axon to Ca^{2+} injury is underscored by the compartmentalized localization of Ca^{2+} -binding proteins to the unmyelinated axon in the nerve fiber layer, but not to the initial axon segment where voltage-gated Na^+ channels predominate (Mojumder et al., 2008).

2.4.3. Unmyelinated axons and light-induced damage

The design of the retina is such that light passing through the optical media of the eye transverses the inner limiting membrane, nerve fiber layer, and the rest of the neural retina before impinging upon photoreceptor outer segments. Photoreceptors themselves have a high concentration of mitochondria, primarily for driving protein synthesis necessary for outer segment renewal, but are shielded from high-energy short wavelength light where it is most concentrated in the central retina by the macular pigment. While RGC axons are not photosensitive *per se*, mitochondria are susceptible to light-induced damage through the formation of free radicals and damage to mitochondrial DNA (Godley et al., 2005).

Mitochondria produce ATP via oxidative phosphorylation, which produces toxic reactive oxygen species (ROS). Normally, mitochondrial machinery is exquisitely adapted to counter ROS formation within the organelle by endogenous antioxidant and repair systems. However, many mitochondrial components that include the enzyme cyclooxygenase (COX) and certain flavin protein nucleotides are highly absorptive of light, particularly the shorter wavelengths within the visible spectrum (reviewed by (Osborne, 2010)). Over time, photointeractions with these components can cause breakdown of the endogenous mitochondrial pathways that counter oxidative stress (Godley et al., 2005). As the nervous system ages, mitochondria become less efficient at balancing ROS formation with metabolic demand (Navarro and Boveris, 2007). Some evidence suggests that RGC exposure to light over time accelerates this process, leading to increased susceptibility to disease processes in optic neuropathies (Osborne, 2010; Osborne et al., 2010).

2.4.4. ATP and axonal neurofilaments

The primary etiology of many optic neuropathies involves genetically-entrained mitochondrial dysfunction (Lee et al., 2010; Nguyen et al., 2011a; Yu Wai Man et al., 2005). In glaucoma, it is not clear to what extent the primary stressors (age and IOP) initiate early mitochondrial-related pathogenic events or whether such events are downstream of remodeling of the nerve head architecture or other factors (Burgoyne, 2011). Mitochondrial motility and maintenance of ATP concentration throughout the axoplasm are critical for normal axonal function. The broad implications of mitochondrial dysfunction for glaucoma have been reviewed extensively elsewhere (Almasieh et al., 2012; Carelli et al., 2002; Lee et al., 2011). Briefly, wherever mitochondrial density is typically high, reflective of higher metabolic demand, susceptibility to mitochondrial- and ATP-related stress will be higher, in particular as it relates to intracellular Ca^{2+} buffering (Sheng and Cai, 2012). Thus, the unmyelinated segment of the RGC axon is particularly vulnerable to stress due to accumulated ATP (Franke et al., 2006; Lee et al., 2011).

Normal aging involves a general reduction in neuronal ATP throughout the brain and other changes associated with mitochondrial stress (Chrysostomou et al., 2010; Lee et al., 2011; Navarro and Boveris, 2007). Mitochondrial transport within the axon, like all transport, relies heavily on available ATP. Diminished mitochondrial transport to distal neuronal processes has been implicated in Alzheimer's and other age-related diseases (Chang and Reynolds, 2006; Ebneith et al., 1998; Rintoul and Reynolds, 2010). Recently, we have shown that in DBA, ATP diminishes in the optic nerve with age; this reduction is exacerbated by elevated IOP,

leading to a decline in the compound action potential (Baltan et al., 2010).

Recent computational modeling indicates that stress due to elevated IOP could challenge axoplasmic flow, thereby inducing focal shortages in ATP along the axon (Band et al., 2009). This could challenge axon function earliest at distal sites along the nerve, which rely upon ATP-fueled mitochondrial transport from the cell body. Though mitochondrial transport is mediated by microtubule and actin filaments (Hollenbeck, 1996), their anchoring at local sites depends upon side-arm interactions with phosphorylated neurofilaments (Sheng and Cai, 2012; Wagner et al., 2003). Cytoskeletal transport of neurofilaments is fastest in the unmyelinated segment of the RGC axon, with a velocity gradient that slows with distance from the retina, probably due to increased phosphorylation (Li et al., 2012). An IOP-induced shortage of ATP could slow neurofilament transport even further, causing not only a shortage of stationary mitochondria at distal sites, but also accumulation of phosphorylated neurofilaments at the myelination transition zone. Indeed, immunolabeling in animal models indicates just such a pattern (Howell et al., 2007b; Soto et al., 2008). Finally, we have shown that axon transport in multiple animal models is challenged first at the most distal sites where RGC axons terminate (Crish et al., 2010). This and related topics are taken up in the next section.

3. Axonopathy in glaucoma

3.1. Axonal transport deficits

Several intrinsic characteristics of the RGC axon likely render it susceptible to injury in glaucoma, independent of the many external factors in the axon's milieu that influence pathogenesis. Indeed, many of the earliest studies focused on the optic nerve head as a nexus of axon pathology, particularly in regard to depleted axoplasmic transport. These have been reviewed extensively before (Almasieh et al., 2012; Burgoyne, 2011; Knox et al., 2007; Whitmore et al., 2005). However, key issues are the timing of axonal transport failure relative to other degenerative events, whether loss of anterograde (retina to brain) and retrograde (brain to retina) transport are concurrent or occur separately, and whether the pattern of transport deficits tell us anything about mechanisms of progression.

3.1.1. Retrograde transport and cell body loss

The ultimate result of glaucomatous degeneration is caspase-dependent apoptotic loss of ganglion cell bodies in the retina, for both rodent and non-human primate models and human tissue (Cordeiro et al., 2004; Garcia-Valenzuela et al., 1995; Kerrigan-Baumrind et al., 2000; Kerrigan et al., 1997; Quigley, 1999; Quigley et al., 1995). The mechanisms that lead to apoptotic cascades in glaucoma are myriad with a large degree of overlap in terms of intracellular cascades (Almasieh et al., 2012; Nickells, 2007).

For RGCs, even acute damage to the axon through axotomy is followed by a period of several days of cell body survival followed by rapid apoptosis (Berkelaar et al., 1994; Levin, 1999; Magharious et al., 2011). Damage to the RGC axon via inflammation in the myelinated segment of the nerve during optic neuritis is often reversible, and vision loss is not permanent (Hickman et al., 2002). Such rescue of function would be impossible should RGC bodies disappear altogether. Similarly, deletion of the pro-apoptotic gene *Bax* in the DBA mouse does not inhibit RGC axon loss in the nerve, but does rescue RGC bodies (Libby et al., 2005b). Conversely, insertion of the Wallerian degeneration slow allele (*Wld^s*), which produces a chimeric protein that protects axons against degenerative injury, slows axonal loss in both the DBA and an inducible rat

model (Beirowski et al., 2008; Howell et al., 2007a). RGC bodies with intact axons remain in these models, but showed signs of early apoptotic injury (Howell et al., 2007a). These results suggest a certain degree of compartmentalization between axonal vs. somatic injury (Whitmore et al., 2005), though not necessarily complete independence of the two processes (Howell et al., 2012a).

Experiments using fluorogold to label RGCs following retrograde transport from central injection sites have been a basic staple of glaucoma research in recent years (Danias et al., 2003; Filippopoulos et al., 2006; Mittag et al., 2000; Vidal-Sanz et al., 2001). However, direct comparisons between transported tracers and immune-labeled RGCs underscore that axonal transport is challenged prior to loss of cell bodies. Thus, RGC counts based on retrograde labeling conducted at the end of the experimental period better reflect the degree of axonopathy than actual soma survival. For example, we and others have shown in the DBA tremendous persistence of the cell body following severe depletion of retrograde axonal transport of fluorogold from the superior colliculus (Buckingham et al., 2008; Jakobs et al., 2005), the primary and most distal site in the rodent RGC projection (Crish et al., 2010). Similarly, in a laser-induced mouse model, RGC bodies visualized by selective markers by far outnumber the fraction of cells labeled retrogradely from the colliculus (Salinas-Navarro et al., 2009; Vidal-Sanz et al., 2012). In this model, IOP is elevated rapidly (6 h post-surgery) and transiently (5–6 days; Salinas-Navarro et al., 2009). While the population of immune-labeled RGCs diminishes slowly over the course of weeks, the number of retrogradely-labeled RGCs is reduced sharply by 1 week (Salinas-Navarro et al., 2009; Vidal-Sanz et al., 2012). A similar progression holds for the same model used in rats, though IOP is elevated a bit longer (Vidal-Sanz et al., 2012). These results contrast with the extremely rapid and severe loss of RGC bodies after NMDA-induced excitotoxic insult (Mittag et al., 2000).

3.1.2. Anterograde transport loss is an early reporter of pathogenesis

The results from inducible rodent models just described would seem to indicate that depletion of retrograde transport occurs rapidly in glaucoma (Vidal-Sanz et al., 2012). However, inducible models often incorporate acute elevations in IOP that are often extreme compared to IOP in human glaucoma. For example, in the mouse and rat studies just described, IOP was increased by over 110% during the short experimental period (Salinas-Navarro et al., 2009; Vidal-Sanz et al., 2012). Discerning small differences in the progression of axonal transport deficits may be difficult with extreme elevations in IOP, since degenerative events may be too compressed to separate distinct outcomes.

In the DBA mouse, progression is spread over several months with modest IOP elevations for many animals as age increases (Inman et al., 2006; Libby et al., 2005a). We and others have found that in the DBA, retrograde transport of fluorogold from the SC to the retina is challenged at intermediate stages but persists quite late into progression, up to 18 months (Buckingham et al., 2008; Danias et al., 2003). In contrast, active anterograde transport of cholera toxin b (CTB) from the retina to the SC and other central targets is challenged much earlier, even by 3 months of age (Crish et al., 2010). Depletion of CTB signal in the SC depends primarily on age, with IOP as an additional stressor, and is eradicated by 11–12 months (Crish et al., 2010; Howell et al., 2012b). At these ages, a substantial number of animals have intact optic nerves (Buckingham et al., 2008; Inman et al., 2006), though this is highly variable between colonies (Howell et al., 2012b; Libby et al., 2005a, 2005b). Similarly, for rats with modest IOP elevations (40–45%) induced by microbead occlusion of the anterior chamber, anterograde transport is diminished for aged but not younger animals

after only 2 weeks (Crish et al., 2010). This is well before overt pathology in the retina or nerve occurs in this model (Chen et al., 2011; Cone et al., 2010; Sappington et al., 2010).

Based on these comparisons, deficits in anterograde transport are an earlier reporter of RGC pathogenesis than failing retrograde transport, with the caveat that the two are usually probed using different tracers. Even so, there may be mechanistic reasons why anterograde transport would be challenged earliest. Axon transport is mediated by two molecular motors that bind microtubules, the largest of the protein filaments comprising the cytoskeleton. Anterograde transport toward the axon terminal is mediated by kinesin motors, while retrograde transport is mediated by dynein; both obtain energy by hydrolyzing ATP diffusing from mitochondria (De Vos et al., 2008). However, while kinesin hydrolyzes ATP at a single binding site and is confined to a single step size of 8 nm, dynein has four ATP hydrolyzing sites and can take step sizes up to 32 nm (Allan, 2011; Schnitzer and Block, 1997; Takahashi et al., 2004). As a result, the architecture of dynein minimizes ATP consumption to provide force appropriate for load capacity and renders the molecule four times more efficient at ATP consumption than kinesin (Mallik et al., 2004). Thus, in glaucoma, anterograde transport is more vulnerable to failure should available pools of axonal ATP diminish.

3.1.3. The progression and retinopathy of distal transport loss

Crish et al. (2010) found that for the DBA, transport of CTB fails first at the most distal site in the projection, where RGC axons terminate in the superficial SC. Subsequent failure of transport follows a distal-to-proximal progression, with deficits next appearing in the LGN followed by the optic tract, nerve and finally the retina. Indeed, in animals with no transported CTB anywhere in the brain, a robust CTB signal exits the eye, penetrates the nerve head, and extends into the nerve itself (see Fig. 5 of Crish et al., 2010). In animals in which CTB signal is absent even in the nerve, a substantial number of RGCs demonstrate intact CTB uptake. Even with complete failure of transport at distal sites, active uptake of CTB remains largely intact; this indicates that failure at distal sites cannot be attributed to a simple blockade in more proximal structures (Crish et al., 2010). A similar distal-to-proximal progression was observed in C57 mice with microbead-induced elevations in IOP over the course of several weeks (Crish et al., 2010).

The superficial SC contains a complete topographical representation of the retina (Drager and Hubel, 1976). We found in the DBA that loss of anterograde transport in the SC follows a sectorial progression, in which deficits progress either caudally or rostrally to adjoin depletion near the optic disk (Crish et al., 2010). An entire caudal or rostral sector is lost before the opposing sector is affected, with the magnitude of sectorial loss increasing with age. We have also described a similar sectorial pattern of transport depletion for mice and rats with microbead-induced elevations in IOP and for rats with laser-induced hypertension (Crish et al., 2010; Lambert et al., 2011). Interestingly, a similar pattern of transport loss in the SC was described recently in rodents with diabetes induced by systemic streptozotocin (Fernandez et al., 2011). Thus, the sectorial nature of transport depletion in distal RGC targets may reflect a general mechanistic feature of how the RGC projection responds to stress regardless of the particular insult.

3.2. Degeneration of the RGC projection

A host of studies using a variety of animal models examine how elevations in IOP influence RGC survival, as assessed either by axonal injury in the optic nerve or by cell body counts in the retina (Morrison et al., 2005). The cumulative results are as diverse as the

models themselves, largely due to variability in the magnitude and duration of IOP elevations but also to the nature of the experimental insult itself (Morrison et al., 2005). Interestingly, very few compare somatic and axonal outcomes directly or track progression over time compared to a functional outcome, such as axonal transport. Those that do make such a comparison indicate that axonal degeneration, i.e., actual loss of axons in the nerve, is sandwiched in between transport dysfunction and final somatic drop-out in the retina. For example, in the study by Lambert et al. (2011), eight weeks of IOP elevation in rats induced a 70% reduction in anterograde transport to the colliculus, an average 33% loss of axons in the nerve, and an average 16% reduction in RGC bodies assessed by immune-labeling that varied by retinal location (Fig. 4). Also in rats, histological indications of axonal dysfunction (e.g., accumulation of phosphorylated neurofilaments and axonal enlargement) occur prior to actual axon loss (Johnson and Cowey, 2000). Studies using non-human primates also indicate a functional deficit followed by axonal degeneration and eventual RGC body loss (Quigley and Addicks, 1980). Finally, in an inducible C57 mouse model, 3 weeks of a 1.7-fold increase in IOP led to a 21% loss of Brn3-labeled RGC bodies, but a 35% loss of axons in the nerve (Zhu et al., 2012). This lends support to the axon-before-cell body progression with modest elevations in IOP.

Results from the DBA mouse are consistent with this pattern of nerve-before-retina as well (Schlamp et al., 2006). Typical phenotypic penetrance of the iris defects underlying elevated IOP in the DBA is about 50% by 10–11 months (John et al., 1998; Libby et al., 2005a, 2005b; Scholz et al., 2008; Sheldon et al., 1995). We have shown at least two indices of axonal dysfunction occur earlier: depletion of anterograde transport to the SC beginning at 3–5 months and diminished compound action potential in the optic nerve by 6 months (Baltan et al., 2010; Crish et al., 2010). The DBA is intrinsically variable, with many animals showing little or no axon degeneration in the nerve as late as 10–11 months (Inman et al., 2006; Schlamp et al., 2006; Scholz et al., 2008). Somatic drop-out in retina is generally later (Buckingham et al., 2008; Zhong et al., 2007). Direct comparisons between axon counts in the nerve and RGC bodies show the former progressing approximately 2-fold faster (Howell et al., 2012b). On the other hand, functional deficits that influence RGC physiology may be detected prior to any axonal or somatic indications of degeneration (Saleh et al., 2007). This may reflect physiological changes in pre-synaptic circuitry or retinal glial function, as discussed above (see Section 2.3).

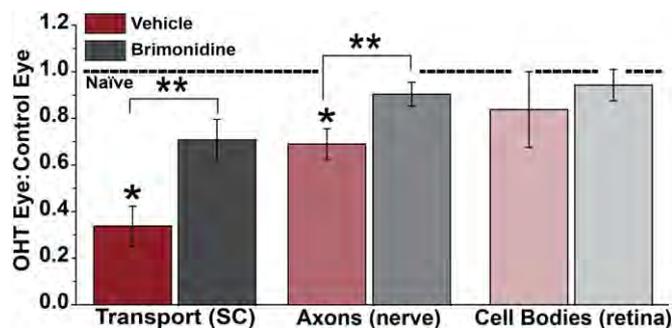


Fig. 4. Distal to proximal progression in an inducible rat model. Three degenerative outcomes after 8 weeks of ocular hypertension (OHT) induced unilaterally in rats by laser cauterization of the episcleral veins. With elevated IOP in the OHT eye, anterograde transport to the SC was affected the worst, with a decrease of 70% compared to the SC from the control eye, followed by a 33% loss of axons in the nerve. RGC body loss was sporadic across the retina, with only a 16% decrease averaged across locations. Protection afforded by systemic treatment with brimonidine was proportionally efficacious. Ratio of unity for the two eyes from naive animals indicated by dotted line. * and ** indicate significance compared to naive ratio or vehicle treatment, respectively ($p \leq 0.02$). Data reproduced from Lambert et al. (2011).

3.2.1. Axon degeneration and IOP

Axon loss in the DBA is related to the magnitude of IOP elevation (Inman et al., 2006; Libby et al., 2005a, 2005b), though other studies have found a weaker relationship, probably due to the large variability in the model (Scholz et al., 2008). In fact, dependency on IOP appears to be a general feature of axonal drop-out in the nerve regardless of how IOP becomes elevated. An elegant series of studies using photocoagulation of the trabecular meshwork in rats described a strong correlation between the degree of axonal degeneration in the nerve and both the cumulative exposure to elevated IOP and the peak IOP (Levkovitch-Verbin et al., 2002). An inducible model in Swiss Black mice also supports a strong correlation between axon loss and cumulative IOP exposure (Mabuchi et al., 2003). The same is so in a non-human primate model (Yucel et al., 2003).

The dependency on IOP holds for a variety of rat models and across magnitudes and duration of exposure. Fig. 5 shows how relative axon degeneration rises with cumulative exposure to IOP, using the thorough synopsis of various rat models provided by Morrison et al. (2005) and augmented with more recent studies. Even for short durations of very high elevations in IOP, axonal degeneration increases linearly with increasing exposure (Chauhan et al., 2002; Chidlow et al., 2011; Dai et al., 2012).

Fig. 5 demonstrates another intriguing trend. When retrograde fluorogold labeling of RGCs is used to assess survival, the dependency on IOP exposure is far less robust. In fact, across the entire 6-fold range of cumulative IOP, the fraction of RGC somatic loss varies only between 10–30%, with very little dependence on IOP exposure. This speaks to several points. The comparison suggests that nerve pathology is *always* greater than that based on RGC counts by a factor of 2–3 regardless of IOP exposure and that nature of the model. This underscores the axogenic nature of IOP-induced injury and the persistence of a large number of RGC bodies throughout progression. By the same token, the same comparison could be interpreted to suggest that for a given IOP exposure, axon degeneration significantly *precedes* loss of retrograde transport. However, most of the fluorogold studies referenced in Fig. 5 applied the tracer

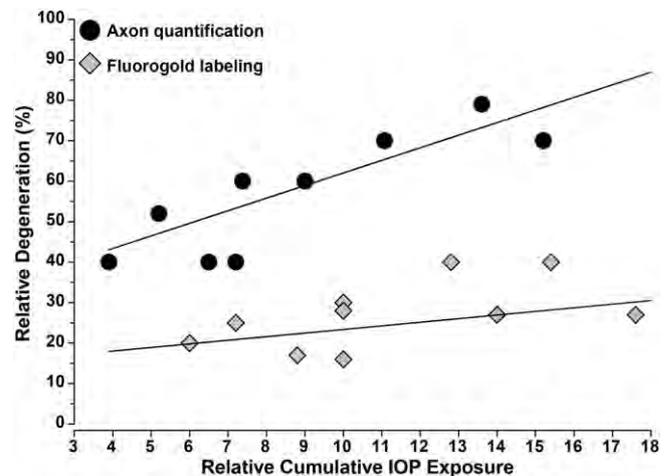


Fig. 5. IOP-induced loss of axons is independent of method. Graph shows relative degeneration assessed by either quantification of surviving RGC axons in the nerve or of retrogradely-labeled RGC bodies in the retina as a function of cumulative exposure to elevated IOP. Data obtained from three independent rat models of ocular hypertension as summarized in Morrison et al. (2005) and supplemented with additional quantification (Jiang et al., 2007; Doh et al., 2010; Munemasa et al., 2010). Relative cumulative IOP exposure calculated as product of fold-increase in IOP above baseline and period of elevation. Axon loss in the nerve increases linearly with IOP exposure independent of model used ($r^2 = 0.77$, $p = 0.004$). Back-filling of RGCs is less informative, with little dependency on IOP exposure ($r^2 = 0.04$, $p = 0.59$).

either prior to or concurrent with IOP elevation as a means to pre-label RGCs (see Table 1 in Morrison et al., 2005). The tacit assumption in such experiments was that drop-out of fluorogold-labeled cell bodies would be the most telling outcome measure, and so the idea was to label them beforehand. This stands in contrast to its use in the chronic DBA model, in which the degree of retrograde labeling is a measure of intact axonal function in the animal's history prior to injection (Buckingham et al., 2008; Danias et al., 2003; Jakobs et al., 2005; Zhong et al., 2007). Indeed, the number of successfully-filled RGCs based on retrograde transport of fluorogold diminishes proportionally as the number of overtly degenerating axon profiles in the nerve increases (Zhong et al., 2007).

The compound action potential (CAP) of the DBA optic nerve also depends strongly on IOP for young animals (6 months), with recovery of the CAP after oxygen–glucose deprivation diminishing linearly with increasing IOP above a certain threshold (Baltan et al., 2010). For older animals (10 months), IOP has little influence over CAP recovery, possibly due to already diminished ATP in the older nerves. However, CAP amplitude, which indicates the fraction of functional axons, in older animals is significantly diminished compared to young; elevated IOP for both groups exacerbates the loss (Baltan et al., 2010). This is interesting, given that depletion of anterograde transport in the DBA also depends primarily on age with IOP as an additional stressor (Crish et al., 2010).

3.2.2. RGC synaptic terminals and central projection sites

Axon degeneration in the nerve is marked by reduction in both axon sampling density (number of axons per unit cross-sectional area) and total number of axons in the nerve. This is so for inducible rodent models (Dai et al., 2012; Morrison et al., 2005), DBA mice (Howell et al., 2012b; Inman et al., 2006; Libby et al., 2005a; Scholz et al., 2008), monkeys (Levkovitch-Verbin, 2004; Quigley et al., 1981, 1987), and of course human patients (reviewed in Quigley, 1999). However, functional studies utilizing active transport markers, whole nerve physiology, and ERG measurements of RGC response indicate an interval between functional impairment and outright degeneration of the optic projection (Baltan et al., 2010; Buckingham et al., 2008; Holcombe et al., 2008; Saleh et al., 2007). Such an interval is also implied by the reversibility of both axonal transport deficits induced acutely and diminishment of RGC signals in the ERG (Johansson, 1988; Minckler et al., 1977; Nagaraju et al., 2007; Porciatti and Nagaraju, 2010; Quigley and Addicks, 1980). A similar phenomenon has been described in human glaucoma, with reversal of physiological deficits subsequent to IOP-lowering surgery (Sehi et al., 2010).

How long is this interval? In the DBA mouse, anterograde transport in the SC is depleted by 11–12 months (Crish et al., 2010; Howell et al., 2012b), but protein markers for RGC presynaptic terminals and synapses persist up to 18–22 months (Crish et al., 2010). This is also consistent with the longer viability of retrograde transport from the SC (18 months: see Section 3.1.2), which requires intact RGC axon terminals for uptake. We also found that SC volume persists for the same period, indicating little or no loss of post-synaptic relay neurons (Crish et al., 2010). Interestingly, King et al. (2006) showed an increase in receptive field size for glaucomatous SC relay neurons in an inducible rat model, supporting the possibility of physiological plasticity prior to degeneration of the projection.

A number of studies in non-human primates show significant shrinkage and loss of RGC relay cells in the LGN as well as reduced cytochrome oxidase activity after several months of induced ocular hypertension (Harwerth et al., 2002; Weber et al., 2000; Yucel et al., 2006, 2003). A similar result was demonstrated in post-mortem tissue for patients with significant (50%) visual field deficits (Gupta et al., 2006). Presumably pathological changes in RGC

projection sites ensue from a form of anterograde trans-synaptic degeneration (Yucel and Gupta, 2008). In support of this, loss of LGN neurons increases as RGC axon degeneration in the nerve progresses, but generally lags by 20–30% (Yucel et al., 2003). For example, even with near 100% axonal degeneration, 30–40% of LGN relay neurons persist (Yucel et al., 2003).

3.2.3. Progression and pattern of degeneration in the nerve

Our studies of the DBA indicate a distal-to-proximal progression in the depletion of anterograde transport from the retina to the SC (Section 3.1.3 above). We also found that axon degeneration in the DBA optic nerve (myelinated portion) is always worse at sites nearer the optic chiasm than the orbit, as assessed by counts of degenerating profiles (Crish et al., 2010). Similarly, Schlamp et al. (2006) showed a distal-to-proximal depletion of tracer transport in the DBA optic nerve, while axonal dystrophies decrease in severity from the SC toward the optic nerve head (Howell et al., 2007a). As discussed by Howell et al. (2012a), this sort of pattern suggests a “dying back” progression of axonal degeneration, which involves the slow advancement of pathology from distal processes toward the cell body (Coleman, 2005). With a dying back program, one might expect substantially more loss of RGC axon terminals with tell-tale formation of end-bulbs at distal projection sites than axonal drop-out even in the distal nerve. However, a full complement of RGC axon terminals persists in the SC long after axon degeneration in the nerve begins (Crish et al., 2010; Inman et al., 2006; Scholz et al., 2008). Furthermore, axonal dystrophies in the SC are not as severe as one might expect from full-blown dying back (Howell et al., 2007a).

The pattern of axonopathy in the DBA nerve when viewed in cross-section mimics the sectorial retinopathy of transport deficits in the SC (Fig. 6). This has been demonstrated by histological staining of axons, which shows degenerating axons forming clusters of affected fascicles or bundles separated by hypertrophic astroglial processes (Schlamp et al., 2006; Scholz et al., 2008). This sectorial progression is also evident in human glaucomatous post-mortem tissue (Knox et al., 2007; Quigley, 1999). Similar patterns in the nerve emerge from immune-labeling for axonal components such as phosphorylated neurofilaments in conjunction with cytoskeletal markers for astrocytes (Howell et al., 2007a; Soto et al., 2008). These experiments suggest that as axons dropout of the nerve, hypertrophic astrocyte processes expand to preserve overall nerve volume by forming a glial scar. In inducible models for rodents, the progression of axon loss is often rapid, depending on the insult (Chidlow et al., 2011; Fu and Sretavan, 2010). This is discussed in detail below (see Section 4.1). Nevertheless, histological staining of axons in these models also reveals a sectorial pattern of degeneration (Morrison et al., 1997; Soto et al., 2011), as does immune-labeling for axonal components (Vidal-Sanz et al., 2012).

3.2.4. The proximal axonal segment

RGC somatic degeneration, like axonal in the nerve, is also sectorial across animal models including DBA (Danias et al., 2003; Jakobs et al., 2005; Schlamp et al., 2006; Soto et al., 2008), inducible mouse and rat (Fu and Sretavan, 2010; Soto et al., 2011; Vidal-Sanz et al., 2012), and monkey (Harwerth et al., 2002). In the proximal portion of the optic nerve head, retinotopy is conserved such that specific retinal regions are represented by neighboring axonal bundles in the nerve. Using a DBA strain with an RGC reporter, Schlamp et al. (2006) demonstrated that regions of axonal degeneration in the nerve map fairly well to regions of the retina lacking RGCs, though pathology in the nerve was always worse. Similarly, using another RGC reporter crossed into the DBA, Howell et al. (2007a) showed that sectors of surviving RGCs in the retina mapped to intact axonal bundles in the nerve head. These results

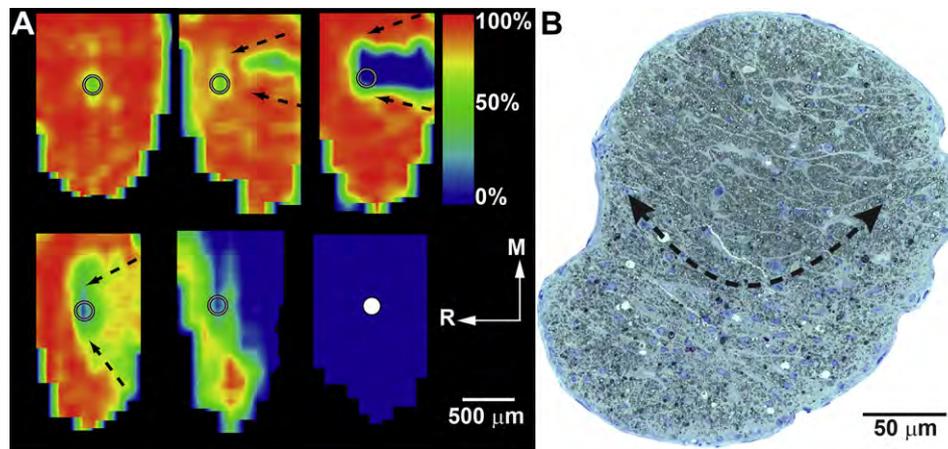


Fig. 6. Sectorial progression in the DBA mouse. **A:** Retinotopic maps of SC from DBA mice reconstructed from serial coronal sections showing levels of cholera toxin B transported from the retina after intravitreal injection. Degree of intact signal density varies from 100% (red) to 50% (green) to 0% (blue). Maps show the representation of optic disk gap (circle) and sectorial progression of deficits in transport increasing in severity from the caudal edge to the optic disk gap (arrows). Ages are (left to right, top to bottom): 3, 3, 8, 10, 10 and 12 months. R: rostral; M: medial. Maps reproduced from Crish et al. (2010). **B:** Cross-section through optic nerve of DBA mouse. Arrows outline a sector with advanced progression containing numerous degenerating profiles and extensive gliosis. The unaffected region of the nerve contains fascicles of intact axons separated by astrocyte processes.

indicate that somatic degeneration follows axonal degeneration not only temporally, but topographically as well.

That somatic and axonal progressions are coupled might, at first blush, appear to contradict the evidence supporting compartmentalized degeneration (see Section 3.1.1.). However, even in the DBA *Wld^s* mouse, those eyes that eventually demonstrate severe axon degeneration in the nerve also show a correlative loss of RGC bodies (Howell et al., 2007a). Thus, loss of the axon even if delayed must, at some point, necessitate loss of the cell body. In the DBA *Bax* knockout, with disease progression the RGC unmyelinated axon survives along with the protected cell body and extends through the level of the glial lamina of the optic nerve head (Howell et al., 2007a). Label for axons in degenerating DBA nerve also extends well past the lamina region (Soto et al., 2008), as do transported fluorescent markers even late in progression (Crish et al., 2010; Schlamp et al., 2006). Were the unmyelinated segment to retract early in chronic glaucoma, one would expect a dominant pattern of dystrophic axons and end-bulbs in the nerve head and optic disk of the pre-laminar region indicative of acute degeneration; this is not the case (Howell et al., 2007a). Even with more acute (3-fold increase) and transient (days) elevations in IOP, tracers applied directly to the proximal nerve posterior to the lamina region label considerably more RGCs than fluorogold retrogradely transported from the SC (Salinas-Navarro et al., 2010; Vidal-Sanz et al., 2012). Thus, the unmyelinated axonal segment, while doubtlessly involved in transducing IOP-related stress at the nerve head, ironically appears to persist along with the cell body after distal axonopathy has progressed considerably.

4. Progression and the nerve head

4.1. Just passing through: the optic nerve head

A major goal has been to understand how factors extrinsic to the RGC axon as it passes through the nerve head contribute to a pathogenic environment and initiate and modulate progression (Hernandez, 2000; Moore and Goldberg, 2010; Osborne et al., 2001; Venkataraman et al., 2010; Vrabec and Levin, 2007). The extra-axonal milieu includes the vascular and glial architecture of the retina and optic nerve head, the scleral–lamina interface, and of course the lamina cribrosa or glial lamina as it is known in rodents (Howell et al., 2007a; Jakobs et al., 2005; Morgan, 2000). Of the

extrinsic modulators of survival that arise from these architectural substrates, it is useful to distinguish those that could transduce *directly* age- and IOP-related stress to the axon – which we might call primary transducers – from those that influence RGC survival but are themselves downstream of IOP- or age-dependent factors. This distinction is addressed in detail within the rubric recently formalized as the “biomechanical paradigm” (Burgoyne, 2011). This elegant paradigm seeks to understand how age- and IOP-related stressors are conveyed to the RGC axon through biomechanical, inflammatory and metabolic signals from the extra-axonal milieu in the nerve head. It links the cytoarchitecture of the nerve head to direct transduction of IOP-related stress on a backdrop of age-related changes in structure and neurochemistry. The paradigm can be no better articulated than it has been by its authors (Burgoyne, 2011; Burgoyne et al., 2005), and so will not be discussed further here.

4.2. Acute injury and the axonal “choke point”

The optic nerve head defines the transition between the unmyelinated RGC axon and the point at which it becomes myelinated by oligodendrocyte glia; this is also an important zone for astrocyte–axon interactions (Dai et al., 2012; Hernandez, 2000; Minckler et al., 1976; Nguyen et al., 2011b; Son et al., 2010). This zone has often been interpreted as an axonal “choke point” at which transport is acutely stressed for induced elevations in IOP exceeding what is typical in chronic models or human glaucoma (reviewed in Knox et al., 2007). Acute stress in such models is typically transient (hours to days) and generally accompanied by rapid axonal degeneration in the nerve and compression of the interval between axon loss and somatic degeneration in the retina (Chidlow et al., 2011; Fu and Sretavan, 2010; Zhang et al., 2009).

The earliest studies describing the lamina region of the optic nerve head as a bottle-neck for antero- and retrograde transport in monkeys incorporated pressure increases of 100–1000% (Minckler et al., 1977; Quigley and Addicks, 1980). For monkeys with less IOP exposure, transport blockade was mostly absent (Quigley and Addicks, 1980). In some cases, transport blockade was reversible (Minckler et al., 1977; Quigley and Addicks, 1980), suggesting that neither RGC bodies nor axons had undergone significant degeneration due to the transport deficit. Axons subjected to very high pressure demonstrate clear morphological indications of

mechanical damage (Quigley and Addicks, 1980; Quigley et al., 1980). Interestingly, like the chronic rodent models, the unmyelinated axonal segment and cell body often persist after overt degeneration of the retrobulbar nerve (Quigley and Addicks, 1980). Transport blockade with acute elevations in the monkey accompany rapid optic disc deformation, accumulation of cytoskeletal proteins in the pre-laminar region, and axonal dystrophies in the proximal nerve and nerve head (Balaratnasingam et al., 2007; Hayreh et al., 1999; Pease et al., 2000). With even higher elevations for several hours, anterograde transport is blocked at the laminar region with accumulation of cytoskeletal organelles and clear axonal dystrophies (Anderson and Hendrickson, 1974). Similar characteristics have been described in human post-mortem nerve from eyes with secondary acute glaucoma (Knox et al., 2007).

In rodents with acute elevations, the story is very much the same, with sharp, temporary increases eliciting reversible mechanical blockage of axoplasmic transport (Johansson, 1988). In rats with IOP acutely raised 3–5 times above normal, retrograde transport of radiolabeled BDNF (brain-derived neurotrophic factor) from the SC to the retina is diminished by 50% and accompanied by axonal dystrophies proximal to the myelination transition zone (Pease et al., 2000). For IOP exposures less than a week that elicit 25–50% axon loss in the rat proximal nerve, protein markers for axonal transport accumulate on either side of the transition zone (Chidlow et al., 2011), suggestive of a locus of acute insult. In contrast to chronic and more modest IOP elevations, axon degeneration with acute elevations can appear worse proximal to the laminar region of the nerve head than it is at distal sites further along the nerve (Beirowski et al., 2008).

4.3. Two forms of progression ... or more?

The distinction just made between acute elevations in inducible models and IOP in human glaucoma is not intended to detract from the importance of such models, which have been seminal in establishing the link between IOP-related biomechanical force at the nerve head and pathogenesis. Taken as a whole, the acute studies support two important conclusions about axonopathy and the optic nerve head. First, for extreme elevations in IOP which lead to dramatic diminishment of ocular perfusion pressure (Anderson and Hendrickson, 1974; Knox et al., 2007; Quigley and Addicks, 1980), mechanical compression of the unmyelinated axon as it transverses the laminar region is the likely mechanism of focal depletion of axoplasmic transport (Quigley and Addicks, 1980; Quigley et al., 1980). Such a scenario could be considered the strong form of the biomechanical paradigm, for it certainly follows from the architecture of the scleral-nerve interface, especially in the primate with its well-defined lamina cribrosa (Burgoyne, 2011; Burgoyne et al., 2005). Second, in contrast to the slower distal-to-proximal progression in the DBA nerve (Crish et al., 2010; Schlamp et al., 2006), acute elevations may induce Wallerian-like degeneration distal to the point of injury. This sort of injury is characterized by early axonal dystrophies followed by rapid disassembly of the axon distal to the injury (Coleman, 2005; Howell et al., 2012a; Whitmore et al., 2005). Interestingly, even in the chronic DBA, axonal dystrophies along the optic nerve are highly suggestive of Wallerian degeneration (Howell et al., 2007a).

Wallerian degeneration has also been linked to another mode of axonopathy termed “acute axon degeneration”, which occurs both distal and proximal to the point of stress (Coleman, 2005). This may explain in some inducible models the presence of dystrophies on either side of the myelination transition zone (Chidlow et al., 2011), as well as early axonal loss in the proximal nerve compared to distal (Beirowski et al., 2008). Aberrant unmyelinated axonal processes observed late in progression in the retina of both the DBA and an

acute model are also suggestive of a separation on the proximal side of the nerve head (Fu and Sretavan, 2010; Soto et al., 2008), although they could represent anomalous sprouting as seen in white matter tracts in Parkinson’s disease (Braak et al., 2004).

We are left with a model in which progression in glaucoma likely involves both early and progressive distal axonopathy with components of more acute Wallerian degeneration (Nickells et al., 2012; Howell et al., 2012a; Whitmore et al., 2005), as summarized below (Fig. 7). It is possible that the balance between the two programs depends on the degree of stress placed on a lynchpin defined by the unmyelinated axon as it passes through the laminar region of the optic nerve head. The transduction of stress directly to the axon is presumably mediated by the constellation of architectural, inflammatory, vascular and other signaling factors included in the biomechanical paradigm (Burgoyne et al., 2005; Burgoyne, 2011). This model would mandate that the more acute the stress, the greater the pressure on the system toward Wallerian degeneration (Howell et al., 2012a). In human patients IOP-related stress is subject to tremendous variability, as in the DBA model (Inman et al., 2006; Schlamp et al., 2006). Thus, Wallerian forces may move in and out of the fore as IOP varies and distal axonopathy progresses.

4.4. Relation to dendritic pruning

A well-known aspect of neuronal degeneration in a variety of diseases is morphological changes in the dendritic arbor, in the form of both dendritic thinning and dendritic loss (Liu et al., 2011). Dendritic stress and the loss of synapses associated with it are potential targets for therapeutic intervention, and so it is important to establish their relation to axonal degeneration (Morquette and Di Polo, 2008). A study using severely diseased human post-mortem tissue found that the few surviving midget and parasol ganglion cells demonstrated a reduction in both dendritic complexity (branching) and dendritic field size (Pavlidis et al., 2003). That both were observed indicates that RGC dendritic arbors do not simply shrink down during pathogenesis while conserving complexity, but actually undergo pruning at distal branch points. Interestingly, in the same study, cell body size was unchanged compared to normal tissue (Pavlidis et al., 2003). This could be interpreted as an indication that dendritic pruning occurs prior to apoptotic changes in the cell body.

A key question is how early dendritic changes occur. The answer based on animal models is not straightforward. In an inducible cat model, substantial dendritic pruning was concurrent with RGC body drop-out in the retina after a month of hypertension (Shou et al., 2003). While average IOP was only about twice that of normal (18 mmHG), the peak IOP was often 20–25 mmHG higher. Similarly, in glaucomatous monkey retina, pruning of dendritic arbors for parasol cells occurred over the course of 2–4 weeks along with shrinkage of cell bodies (Weber et al., 1998). IOP in this study involved elevation 5–7 times normal with peaks near 90 mmHG. Later studies in monkeys examined dendritic changes months and even years after injury (Weber and Harman, 2005). Importantly, in the monkey model most affected RGCs retained at least the proximal axon segment.

In the DBA retina, severe progression is marked by sectors containing far fewer identifiable RGC bodies (Jakobs et al., 2005). Surviving RGCs in these sectors have fewer dendritic branch points, smaller cell bodies, and thinner axons, although whether these features always occur together is not established. Some cells have tortuous dendritic arbors suggestive of remodeling (Jakobs et al., 2005). The same study showed that RGCs with dramatic dendritic or somatic shrinkage are never filled by retrograde label from the superior colliculus. That these cells retain partial dendritic arbors

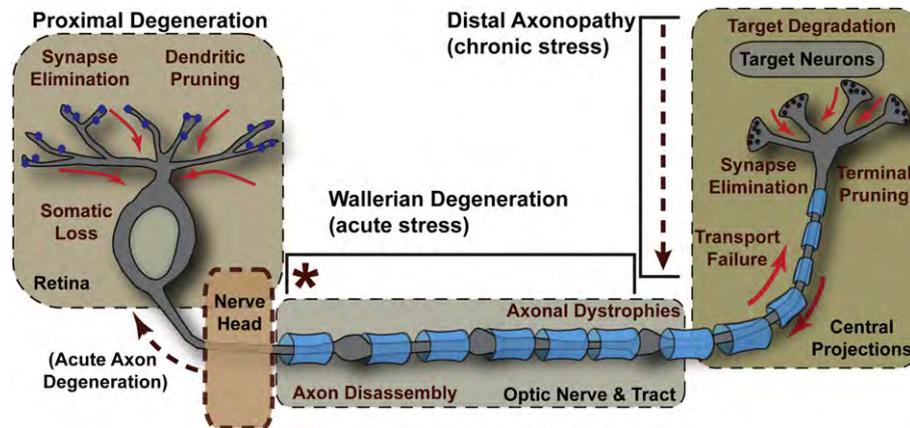


Fig. 7. Key events in progression. The events defining RGC degeneration in glaucoma span four critical regions: retina, optic nerve head, optic nerve and tract, and the central projection to the brain. Disease-relevant stress originating in the nerve head (marked by *) induces a program of distal axonopathy with several characteristics of chronic progression. These include failure of intra-axonal transport and subsequent loss of RGC pre-synaptic active zones and axon terminals in central projection sites, followed by degeneration of target neurons. Pathogenic features follow a distal-to-proximal progression, as in “dying back” neuropathies. In the optic nerve and tract, degeneration of the myelinated RGC axon (indicated by blue oligodendrocyte sheaths) includes features of both distal axonopathy and Wallerian degeneration, including axonal dystrophies and more acute axon disassembly beginning distal to the nerve head. Proximal degeneration is marked by elimination of synapses to RGCs, dendritic pruning and eventual somatic drop-out in the retina. In some models, retraction of the unmyelinated segment from the nerve head could arise from acute axon degeneration (see Coleman, 2005).

indicates that dendritic pruning in the DBA occurs either after or concurrent with failure of retrograde transport but before final apoptotic elimination of the cells. Also in the DBA, moderate progression is characterized by upregulation of C1q, the initiating protein in the classical complement cascade, with localization proximal to synapses in the inner plexiform layer (Stevens et al., 2007). The implication of this finding is that synapses to RGC dendrites may be targeted in progression for microglial-mediated elimination. In the DBA, microglia activation is early, and suppression of this response is protective of RGCs (Bosco et al., 2008, 2011).

Thus, it is likely that acute elevations in IOP (as in the monkey model) induce a Wallerian-like rapid degeneration of dendrites concurrent with somal shrinkage and subsequent apoptosis. There is precedence for such a program in other neurons (Tao and Rolls, 2011). However, chronic stress as in the human disease and the DBA appears to lead to a slower pruning of the dendritic tree that seems to precede changes in the cell body that would be indicative of apoptotic processes. This raises the intriguing possibility that like axonal degeneration, dendritic degeneration is to some degree compartmentalized in glaucoma.

5. Epilogue: gaps in our knowledge and future directions

5.1. Transduction of IOP stress

Our view of glaucoma has evolved considerably in recent years, from a disease involving mechanical injury due to elevated IOP to a disease involving a confluence of age- and IOP-related stressors acting upon a neural substrate. Glaucoma is largely axogenic, like other CNS diseases (Coleman, 2005; Whitmore et al., 2005), with axonal dysfunction in the optic projecting occurring prior to outright degeneration and loss of tissue (Fig. 8). This progression could explain why, in non-human primates, significant loss of visual function occurs with only minor changes in the numbers of RGC bodies in the retina (Harwerth et al., 2002). This interval defines an obvious window of opportunity for neuroprotective therapeutic intervention, especially for those patients who do not respond to hypotensive regimens or other treatments, especially for secondary glaucomas. It is therefore critical to focus our energy on understanding the molecular mechanisms through which glaucomatous stressors induce loss of function early in progression.

The unmyelinated segment of the axon both in the retina and in the nerve head is especially vulnerable to disease-related stressors from a metabolic standpoint. This vulnerability is likely to affect distal axonal processes. Additionally, the extracellular milieu of the axon as it passes through the nerve head is likely to become pathogenic. This is so for two primary reasons. First, a confluence of age-related factors diminishes the architectural capacity of the nerve head for IOP-related stress (Burgoyne, 2011). Second, glial and vascular components create an environment that is increasingly inhospitable for the axon through biochemical, metabolic and inflammatory signaling (Almasieh et al., 2012; Hernandez, 2000). Thus, ironically, the very segment of the RGC axon that is likely most intrinsically vulnerable to stress is also poised to *directly* transduce stress from the extra-axonal environment.

We understand very well possible ramifications of this transduction. For example, as discussed above (Section 4.2), one consequence of disrupted axonal transport is deprivation of BDNF-mediated trophic support to RGCs from the SC (Pease et al., 2000; Quigley et al., 2000). Though strong evidence for this hypothesis arises from models employing extreme elevations in IOP, early reduced transport is a fact – and so it is reasonable to conclude that reduced trophic support is as well. Reduced axonal transport is likely to affect a host of other factors as well, including mitochondrial motility and ATP availability (Section 2.4.4).

However, setting aside mechanical compression, we do not understand how IOP-related stress is transduced from cellular structures in the nerve head to the axon to cause early transport deficits. Astrocytes are likely to be important mediators in this transduction pathway (Hernandez, 2000; Morgan, 2000; Son et al., 2010). One possibility (of many) is astrocyte release of the dually neuroactive and vasoactive peptide endothelin-1 (Rosenthal and Fromm, 2011). Cultured optic nerve astrocytes release endothelin-1 in response to tumor necrosis factor α (TNF α : Desai et al., 2004), which is also upregulated in the glaucomatous nerve head and contributes to axonal degeneration (Nakazawa et al., 2006; Tezel, 2008). Endothelin-1 is known to impede anterograde and retrograde transport in RGC axons, possibly by increasing intra-axonal Ca^{2+} (Stokely et al., 2002; Wang et al., 2008b), which in turn may disrupt the axonal cytoskeleton (Crish and Calkins, 2011). Another possibility suggested by morphological changes in the nerve head is withdrawal of astrocyte-derived metabolic support of the axon (Dai et al., 2012).

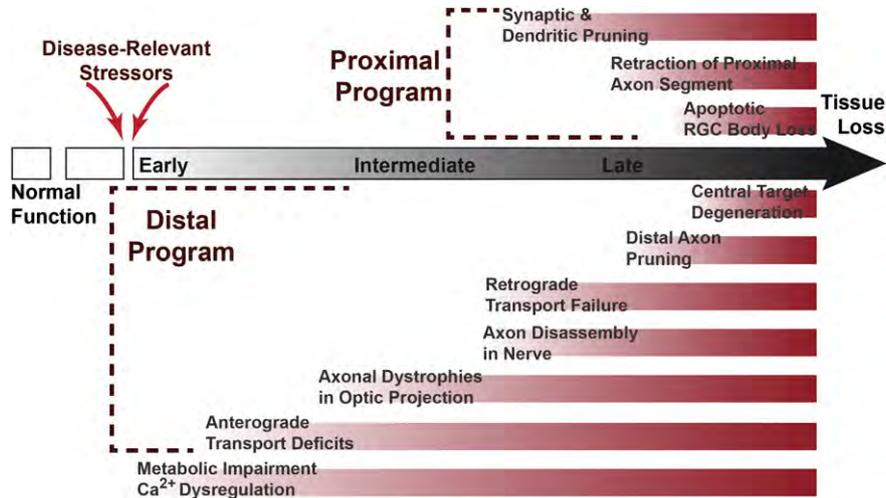


Fig. 8. Proposed timeline of key events in RGC degeneration. Normal function in the optic projection is interrupted by disease-relevant stressors, inducing RGC metabolic impairment and dysregulation of intracellular Ca^{2+} . An early consequence is disruption of anterograde axonal transport at distal sites in the optic projection followed by formation of axonal dystrophies. Retrograde transport persists longer in progression and is likely to fail concurrent with axon disassembly. Later in progression, proximal degeneration affects RGC processes in the retina, though the exact timing of synaptic and dendritic pruning is unknown. In this proposed model, the last events are RGC somatic loss proximally and degeneration of neurons in central targets distally, resulting in irreversible tissue loss. Aberrant physiological signaling in the retina and projection likely is early and progressive.

An intriguing possibility is that RGCs themselves also sense stress-related changes in their environment. We have shown that RGCs express the transient receptor potential vanilloid-1 subunit (TRPV1) in both the dendritic arbor and the unmyelinated axonal segment and that this channel gates a potent Ca^{2+} conductance in response to stress (Sappington et al., 2009). The TRPV1 channel responds to a variety of insults in the CNS and may mediate pathways that counter reduced function (Ho et al., 2012). RGCs also express purinergic receptors that respond to autocrine release of ATP following mechanical stress (Xia et al., 2012). Such a system could counter diminished availability of ATP in the local milieu during progression.

5.2. Mediation of sectorial progression

We have discussed herein how aspects of RGC degeneration in both the distal projection and in proximal structures follow a sectorial progression, filling in from one retinotopic region to the next. This progression is analogous to the pattern of visual field deficits in both human glaucoma and in non-human primate models (Harwerth et al., 2002). While it is tempting to consider this pattern etiologically-specific to glaucoma, other common optic neuropathies demonstrate sectorial deficits as well (Bernstein et al., 2007a, 2007b; Kedar et al., 2011), as does an animal model of streptozotocin-induced diabetes (Fernandez et al., 2011). The latter is especially interesting since the insult is systemic. Thus, retinotopically-defined progression seems to be a general feature of RGC degeneration that transforms a diverse array of disease-specific stressors into a common pathogenic arm.

What such an arm might comprise is unknown. Axons in the optic nerve cluster into well-defined fascicles separated by astrocyte processes. In the proximal nerve, these form a loose retinotopic map, supporting the idea that sectorial progression is axogenic, involving both inter-axonal and axon-astrocyte signaling. In glaucoma, early field loss is modest and first appears as a discrete scotoma before progressing throughout a retinotopic quadrant (Iester et al., 2012). A key question is whether a pathogenic sector begins as a single locus that expands or as several discrete foci within a local region that eventually coalesce as a solid lesion. Once a lesion begins, it is important to understand the processes that confine the injury to a particular sector. Thus, new investigations

must focus not only on the mechanisms of progression within a sector, but on possible mechanisms that sequester the injury to delay spreading between sectors.

5.3. Self-repair pathways

Other retinal neurons are only moderately susceptible in glaucoma to trans-synaptic degeneration. Whatever loss that does occur does so in varying degrees and with high variability between studies and models. This loss is also retinotopically coincident with regions of high RGC damage (Section 2.3.3). However, given that RGCs are so robustly targeted in the disease through processes that include early functional deficits, it is most puzzling that trans-synaptic degeneration of their pre-synaptic circuits is not far greater than it is.

One conjecture to explain this paucity is that even late in progression, RGCs maintain a certain degree, however minimal, of residual physiological activity that drives the maintenance of pre-synaptic connectivity. We found using the Ca^{2+} surrogate manganese coupled with magnetic resonance imaging very little change in cationic activity in the DBA inner retina for ages associated with axonopathy (Calkins et al., 2008). This would seem to speak against early loss of synaptic activity that would be required by dendritic pruning. Similar, a rarely cited study using rats found that modest IOP elevations were associated with *expanded* RGC physiological receptive fields, possibly attributable to enlargement of their dendritic arbors (King et al., 2006).

Perhaps pruning of RGC synapses and dendrites in the inner retina is far slower than predicted by acute models, which likely do not recapitulate the more measured progression in the human disease. Another possibility is that despite ongoing targeting of RGC synapses and dendrites by disease processes, excitatory RGC activity persists because of intrinsic mechanisms for self-repair that counter the degenerative cascade to maintain connectivity with presynaptic circuits. The potential for such a self-repair pathway is evident in experiments demonstrating the utility of exogenous electrical stimulation for promoting RGC survival (Chang and Goldberg, 2012). An exciting possibility is that self-repair pathways may also operate distally, promoting the persistence of RGC axon terminals at their central projection sites even well-after the depletion of anterograde signaling from the retina (Crish et al., 2010).

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