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# Adaptive Responses to Neurodegenerative Stress in Glaucoma

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## Abstract

Glaucoma causes loss of vision through degeneration of the retinal ganglion cell (RGC) projection to the brain. The disease is characterized by sensitivity to intraocular pressure (IOP) conveyed through the optic nerve head, through which RGC axons pass unmyelinated to form the optic nerve. From this point, a pathogenic triumvirate comprising inflammatory, oxidative, and metabolic stress influence both proximal structures in the retina and distal structures in the optic projection. This review focuses on metabolic stress and how the optic projection may compensate through novel adaptive mechanisms to protect excitatory signaling to the brain. In the retina and proximal nerve head, the unmyelinated RGC axon segment is energy-inefficient, which leads to increased demand for adenosine-5′-triphosphate (ATP) at the risk of vulnerability to $Ca^{2+}$-related metabolic and oxidative pressure. This vulnerability may underlie the bidirectional nature of progression. However, recent evidence highlights that the optic projection in glaucoma is not passive but rather demonstrates adaptive processes that may push back against neurodegeneration. In the retina, even as synaptic and dendritic pruning ensues, early progression involves enhanced excitability of RGCs. Enhancement involves depolarization of the resting membrane potential and increased response to light, independent of RGC morphological type. This response is axogenic, arising from increased levels and translocation of voltage-gated sodium channels (NaV) in the unmyelinated segment. During this same early period, large-scale networks of gap-junction coupled astrocytes redistribute metabolic resources to the optic projection stressed by elevated IOP to slow loss of axon function. This redistribution may reflect more local remodeling, as astrocyte processes respond to focal metabolic duress by boosting glycogen turnover in response to axonal activity in an effort to promote survival of the healthiest axons. Both enhanced excitability and metabolic redistribution are transient, indicating that the same adaptive mechanisms that apparently serve to slow progression ultimately may be too expensive for the system to sustain over longer periods.

### Keywords

glaucoma; neurodegeneration; axon degeneration; astrocytes; metabolic stress; oxidative stress; adaptive remodeling; gap junctions

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# 1. Introduction

## 1.1. Glaucoma and IOP

Glaucoma is a group of optic neuropathies that together represent the world's principal cause of irreversible blindness – that is, permanent blindness arising from degeneration of neural tissues in the optic projection from retina to brain. Incidence of glaucoma is increasing as the population ages; approximately 112 million will be afflicted worldwide by 2040 (Tham et al., 2014). Vision loss in glaucoma follows a characteristic pattern of field deficits that progress in wedges from one retinotopic sector to the next (Elze et al., 2015). These deficits are linked to sensitivity to intraocular pressure (IOP), which reflects the production and drainage of aqueous fluid in the anterior eye. This sensitivity is the hallmark feature of glaucoma, which we probe experimentally through induced elevations in IOP using animal models ranging from mice to non-human primates (Bouhenni et al, 2012). We elevate IOP in such models to identify mechanisms that are sensitive to IOP and determine whether modulating their activity or expression, either pharmacologically or genetically, slows progression in one or more models. In doing so we attempt to distinguish elements that are truly causal versus those that result secondarily from neurodegeneration. Importantly, models rarely capture variation in types of glaucoma and in afflicted patient populations, both of which lead to great diversity in IOP and in response to treatment (Susanna et al., 2015).

Glaucoma often (but not always) includes morphological changes in the appearance of the optic nerve head that may indicate nerve atrophy along the perimeter of the optic disc. These changes are associated with optic nerve degeneration but are not necessarily unique to glaucoma (Piette and Sergott, 2006). Rather, they likely reflect a collection of pathophysiological factors that biomechanically influence the structure and morphology of the posterior segment in glaucoma (Burgoyne, 2011). Besides age and IOP, other prominent risk factors are race, severe myopia, central corneal thickness, and familial history along with several gene variants linked to either congenital glaucoma or to greater susceptibility to the disease (Coleman and Miglior, 2008; Weinreb et al., 2014).

The diversity of risk factors for glaucoma speaks to the etiological complexity of the disease. Glaucoma comprises several types that each in some way involve IOP. The different forms of glaucoma tend to fall into two main categories depending on the geometry of the iridocorneal angle where the iris and cornea meet (King et al., 2013). In open-angle glaucoma, the angle's width allows normal outflow of aqueous fluid from the anterior chamber to the drainage canals in the trabecular meshwork. Primary open-angle glaucoma (or POAG) is the most common type and is associated with increasing resistance within the aqueous outflow pathways. POAG encompasses a large IOP range and includes a large proportion of what is known as normal pressure (or normal tension) glaucoma, which occurs in the absence of elevated IOP. The caveat on this point is that some normal pressure glaucoma may present with an etiology separate from that of POAG, involving one or more autoimmune components (Rieck, 2013). In closed-angle glaucoma, narrowing or obstruction of the outflow path challenges drainage of aqueous fluid, which can lead to acute elevations in IOP or even severe ocular pain.

The relative incidence of glaucoma varies widely from population to population, reflecting variation in genetic and other factors that influence anterior segment morphology and susceptibility (Coleman and Miglior, 2008; Weinreb et al., 2014). For all types of glaucoma, clinical treatments attempt to lower IOP through a regimen of topical hypotensive drugs, surgery to facilitate aqueous fluid outflow, or both. Despite this investment in time and resources, many patients continue to lose vision, though there is great variability in the number of estimated non-responders across various studies (Susanna et al., 2015). For patients whose optic projection continues to degenerate despite efforts to lower IOP, we can do little. That we do not understand fully the mechanisms by which sensitivity to IOP exerts its destructive influence emphasizes the need for new therapies that target neurodegeneration directly, whether by protection, repair or outright regeneration (Calkins et al., 2017). Our hope is that such therapies will arise by pinpointing ever-earlier events and markers for progression within the optic projection that could underlie sensitivity to IOP and predispose the system to degeneration (Beykin et al., 2020). Importantly, glaucoma is not linear – the same level of IOP may not have the same influence on the optic projection at different points in progression, even within the same patient. In any case, the discussion of neurodegeneration that follows is agnostic in terms of anterior segment etiology – it focuses on what comes after the threshold for IOP-related pathogenesis is reached – with the following caveat. This review focuses on progressive neurodegeneration, which is typically associated with POAG, not on acute injury such as might arise from sharp IOP elevations or dramatic changes in systemic blood pressure or flow to the eye.

### 1.2. Neurodegeneration in Glaucoma is Bidirectional

The optic projection is formed by the 1.5 million or so axons of retinal ganglion cell (RGC) neurons. Due to the necessity of transparency for the path of light, RGC axons are unmyelinated in the retina and remain so in exiting the eye and entering the pre-laminar region of the optic nerve head (Figure 1). There, the axons form well-defined bundle-like fascicles that penetrate a dense plexus of capillaries, connective tissues, and ramifying processes of astrocyte glia. In forming the optic nerve, these fascicles pass through the lamina cribrosa, which is a porous, multi-layered collagenous structure that couples to the peripapillary sclera (Girard et al., 2011; Quigley, 2015). Because of their architectural orientation and support they provide through interactions with the scleral wall these collagen layers are often called “beams”. Astrocyte processes line the cribrosa pores and, with the cribrosa cells themselves, form a glial membrane that sheathes the passing axon fascicles. In leaving the lamina cribrosa RGC axons finally become myelinated in the retrolaminar region through interactions with oligodendrocytes, as the collection of fascicles form the optic nerve proper. The rodent optic nerve lacks a lamina cribrosa but instead demonstrates a specialized glial lamina in which astrocyte processes ramify transversely to form a honeycomb-like structure of tubes through which unmyelinated axon bundles pass (Sun et al., 2009; Wang et al., 2017). Like the retrolaminar region in humans and non-human primates, RGC axons become myelinated in a transition zone posterior to the glia lamina. Both the central retinal artery and central retinal vein penetrate the disc of the optic nerve head, serving the surrounding tissues through a network of small lateral capillaries.

The optic nerve head is a critical site of pathogenesis in glaucoma, as studies using non-human primates and other mammals with a lamina cribrosa, human donor tissue, and non-invasive imaging of patients have demonstrated. There, multiple neuronal, vascular, glial and biomechanical components influence sensitivity of the optic projection to IOP and its susceptibility to degeneration for a given IOP (Sigal and Ethier, 2009; Burgoyne, 2011; Girard et al., 2011; Quigley, 2015; Wareham et al., 2021). This confluence means that the threshold for IOP-related damage will vary between patients and across different ages. Factors related to blood flow regulation and systemic inflammation in glaucoma influence the biophysical and physiological properties of the cells and tissues that comprise the nerve head. These properties and how they change with aging contribute to the accumulated effects of strain in connective tissues, diminished nutrient diffusion from capillaries to axon bundles, and variation in the capacity of the optic nerve to handle glaucomatous stress and therefore its sensitivity to IOP (Burgoyne, 2011; Downs, 2015).

From the nerve head, RGC degeneration in glaucoma progresses both in the anterograde direction towards central projection sites and in the retrograde direction back to the cell body and dendritic arbor in the inner retina (Figure 2). In the anterograde direction, the optic nerve projects to several subcortical structures, the most distal of which is the superior colliculus (SC). In rodents, nearly all RGC axons project to the colliculus with collaterals extending to the other more proximal nuclei. In primates and many other mammals, the lateral geniculate nucleus (LGN) is the primary projection; in all mammals, the LGN forms the direct projection into the primary visual cortex. An early marker of progression in rodents is degradation of anterograde axonal transport from the retina to central brain projection sites, followed by disassembly of the myelinated axon and degeneration of post-synaptic targets (Crish et al,, 2010;Calkins and Horner, 2012; Calkins, 2012; Crish and Calkins, 2015). In the retrograde direction, RGC dendritic arbors lose complexity as excitatory synapses are eliminated in a complement-dependent process (Agostinone and Di Polo, 2015; Berry et al., 2015; Williams et al., 2016). Pruning due to acute axonal injury in rodents involves loss of activity from mammalian target of rapamycin (or mTOR), which may have bearing in human glaucoma (Agostinone et al., 2018). Multiple types of RGC across vertebrate species reflect the diversity of functional and behavioral specialization. Susceptibility to dendritic pruning likely depends on RGC type, although variability between studies in mice is considerable (El-Danaf and Huberman, 2015; Ou et al., 2016; Risner et al., 2018).

The nerve head is an important nexus for other reasons. As distal (anterograde) and proximal (retrograde) degenerative programs unfold, the RGC body persists much further into progression, long after axon disassembly in the optic nerve and pruning of the dendritic arbor in the retina (reviewed in Calkins, 2012). Importantly, the unmyelinated axon segment also remains intact along with the RGC body, a fact appreciated decades ago from post-mortem histological examination of the human glaucomatous nerve head (Vrabec, 1976). More recently, specific transgenes in mouse models that ameliorate either axonal (*Wlds*) or somatic (*Bax*−/−) degeneration demonstrate the persistence of the unmyelinated segment and that distal and proximal degenerative programs progress to some extent independently (Schlamp, et al, 2006; Beirowski et al., 2008; Howell et al, 2007, 2013). Thus, the junction in the nerve head between the unmyelinated and myelinated axon segments represents a

critical locus in progression. The following section examines physiological properties that may contribute to its importance in glaucoma.

## 2. Axonal Metabolism

### 2.1. Axons are Expensive; RGC Axons are Worse

The visual system is highly energetically expensive (Niven and Laughlin, 2008; Wong-Riley, 2010). Levels of adenosine-5′-triphosphate (ATP) are actually higher in retina than in other prominent brain regions (Zhu et al., 2020). Axons in particular are metabolically demanding. They require a large supply of mitochondrial-generated ATP along their length from cell body to terminals. This supports anterograde transport of mitochondria and synthesized proteins from the cell body to axon terminals, retrograde transport from the terminals back to the cell body, the generation and propagation of action potentials to signal post-synaptic neurons, and reestablishment of the resting membrane potential (Harris and Attwell, 2012; Misgeld and Schwarz, 2017). Excellent recent reviews highlight the metabolic challenges facing the RGC in health and disease (Yu et al., 2013; Osborne et al., 2016; Inman and Harun-Or-Rashid, 2017; Casson et al., 2020).

The capacity to generate action potentials requires not only ATP, but a high density of voltage-gated sodium channels, or NaV, whose distribution along the axon tracks well with that of the mitochondria that provide them energy (Barron et al., 2004). Axons normally transport mitochondria to regions of high metabolic demand. Where axons are myelinated, both mitochondria and NaV cluster at nodes of Ranvier, which support saltatory conductance of action potentials over great distances. While lack of myelination for RGC axons in the retina is beneficial from the standpoint of optics, transparency comes at a price. Unmyelinated axon segments are far less efficient at maintaining ionic gradients necessary for propagating action potentials, about 10-fold less so than the myelinated segment (Perge et al., 2009). Due to the absence of saltatory conduction, the unmyelinated segment requires far greater energy. Thus, the unmyelinated portion of the RGC axon contains a greater abundance of both mitochondria and NaV subunits, as well as the mitochondrial enzymes COX (cytochrome c oxidase) and succinate dehydrogenase, necessary for ATP production (Andrews et al., 1999; Balaratnasingam et al., 2009). As they course through the nerve fiber layer of the retina on their way to the optic nerve head, RGC axons contain intermittent varicosities that are packed with mitochondria and mark points of inter-axonal and axonal-astrocyte contact (Wang et al., 2003). These also contain NaV subunits (Figure 3). In the nerve fiber layer and prelaminar nerve head, the RGC and its axon contain most of the mitochondria, unlike the optic nerve where astrocytes appear to be most rich (Perge et al., 2009).

The caliber of the RGC axon does not increase at the transition between unmyelinated to myelinated segment, as it does for other axons (Perge et al., 2012). Rather, the ganglion cell axon is uniformly thin. To illustrate this point, though the unmyelinated segment is about 50-fold longer than the cone photoreceptor axon its mean diameter is only half (Perge et al., 2012). Thus, the difference of mitochondrial and NaV density between the unmyelinated and myelinated segment does not reflect a bottleneck at the nerve head, such as would occur at the transition from a narrow to wide opening, but rather constitutive differences in metabolic

demand and therefore distribution (Bristow et al., 2002; Yu-Wai-Man et al., 2011). Indeed, like other small axons (Bechtold et al., 2005), thinness places the RGC axon at a natural disadvantage in conditions of metabolic stress due to low capacity to buffer of intraaxonal $Ca^{2+}$ (Stys, 2004; Reeves et al., 2012), even more so for the unmyelinated segment due to higher need for mitochondrial ATP and $Ca^{2+}$ buffering (Franke et al., 2006; Lee et al., 2011).

### 2.2. RGC Axonal Stress Challenges the Limited Supply of ATP

Levels of ATP in the optic nerve directly reflect its capacity to transmit action potentials (Trevisiol et al., 2017). This activity promotes signaling to post-synaptic neurons, which is necessary for focal secretion and retrograde transport of brain-derived neurotrophic factor (BDNF; Lu, 2003; Lou et al., 2005). Axons challenged by stress or injury require increased levels of ATP to support transport of proteins necessary for remodeling and maintenance of cytoskeletal architecture (Her and Goldstein, 2008; Bradke et al., 2012; Zhu et al., 2020). Where myelin is damaged or lost, local stationary mitochondria increase to compensate for diminished pools of ATP (Kiryu-Seo et al., 2010). The rapidity of this response is illustrated aptly for RGCs maintained in culture, in which acute (induced) axonal stress diminishes mitochondrial transport, with recovery of transport linked to greater cell survival (Yokota et al., 2015).

Even with mitochondrial compensation, chronic neuronal stress leads to reduced ATP and increased mitochondrial volume, both of which reduce motility in both anterograde and retrograde directions along microtubule and actin molecular tracks (Chang and Reynolds 2006). Diminished ATP in axon projections greatly reduce active transport and the capacity to maintain microtubule structures within the axon skeleton (De Vos et al., 2007, 2008; Prokop, 2013). The mitochondrial capacity of axons in the central nervous system reduces significantly during aging (Boumezbeur et al., 2010), which may contribute to the age-related loss of axons in the optic nerve that occurs for all mammalian species (Calkins, 2013).

Nerve aging and its associated bioenergetic ramification also influences glaucoma. A useful progressive animal model of glaucoma that has proven convenient for studying RGC axon transport is the DBA/2J mouse (Crish et al., 2010). This inbred strain demonstrates age-related elevations in IOP that correlate with RGC axon degeneration in the optic nerve (Inman et al., 2006; Cooper et al., 2016). In this model age reduces optic nerve mitochondrial COX (cytochrome c oxidase), necessary for ATP production, ATP itself, axon transport, and signaling capacity as measured by the compound action potential; elevated IOP exacerbates these reductions (Baltan, Inman et al. 2010). Similarly aging diminishes mitochondrial transport in non-glaucomatous mouse optic nerve and lengthens axonal segments depleted of mitochondria; induced elevations in IOP also accelerate these effects (Takihara et al., 2015).

Reduced mitochondrial motility and transport to distal neuronal processes contributes to pathogenesis in Alzheimer's and other neurodegenerative diseases (Ebneth, Godemann et al. 1998, Chang and Reynolds 2006, Rintoul and Reynolds 2010). In rodent models of glaucoma, degradation of RGC axonal anterograde transport to central brain targets is an early harbinger of axon dysfunction, which precedes axon disassembly (Calkins, 2012;

Cooper et al., 2020). As with depletion of ATP, age is the predominant factor influencing transport loss with IOP serving as an additional stressor (Crish et al, 2010; Fiedorowicz et al., 2018). Figure 4 summarizes data from the DBA/2J mouse model to demonstrate this point. Transport fails first within the distal-most termination in the SC, with degradation progressing distal-to-proximal from that point in the projection (Crish et al., 2010; 2013; Crish and Calkins, 2015; Ward et al., 2014). This pattern in which distal sites are affected first seems to be a general property of axon injury (Wang et al., 2012). As axons struggle, cytoskeletal transport of neurofilaments needed for repair becomes increasingly important. Transport is fastest in the unmyelinated axon segment and slows with distance from the retina (Li, Jung et al. 2012). Reductions in ATP likely lead to observed accumulations of phosphorylated neurofilaments at the myelination transition zone (Howell et al., 2007; Soto et al., 2008).

Age-related loss of anterograde transport in the DBA/2J is concomitant with malformed and reduced mitochondrial cristae and oxidative capacity and increased mitophagy (Ju et al., 2008; Coughlin et al., 2015; Kleesattel et al., 2015); enhancing mitochondrial function systemically reduces axon degeneration and prevents transport loss (Ju et al., 2010; Harun-Or-Rashid et al., 2018). The pattern of progressive failure in anterograde transport reflects prior depletion of metabolic resources available to the RGC axon, the necessity of intact mitochondrial motility, and the higher metabolic expense of anterograde vs. retrograde transport, which persists as long as the RGC axon itself (Calkins, 2012; Kleesattel et al., 2015; Inman and Harun-Or-Rashid, 2017).

### 2.3. Metabolic and Oxidative Stress: Two Sides of the Same Coin

Depletion of metabolic resources and the RGC intrinsic adaptation to counter this depletion is linked to two inter-related pathogenic arms. The first involves dysregulation of $Ca^{2+}$ homeostasis (Crish and Calkins, 2011; Wang et al., 2012; Sappington et al., 2015), while the second relates to mitochondrial oxidative stress (reviewed in Tezel., 2006; Wareham and Calkins, 2020; Wareham et al., 2021). Diminished ATP and concurrent loss of signaling capacity in the glaucomatous optic nerve includes failure of $Na^+/K^+$ ion pumps (Ames, 2000). As the $Na^+/K^+$ ion exchange fails, $Ca^{2+}$ accumulates within the axon as increased axoplasmic $Na^+$ promotes reverse operation (Stirling and Stys, 2010). This imbalance leads to increased $Ca^{+2}$-dependent protease activity, which degrades the axonal cytoskeleton (Mattson, 2007; Knöferle et al., 2010; Crish and Calkins, 2011). These effects are compounded by diminished anterograde transport of cytoskeletal components to distal axon segments (Her and Goldstein, 2008; Bradke et al., 2012; Zhu et al., 2020). Dysregulation of axonal $Ca^{2+}$ is a major contributor to axonal disassembly and reducing excessive $Ca^{2+}$ is efficacious in slowing degeneration in a variety of conditions (Wang et al., 2012; Vargas et al., 2015). This includes experimental glaucoma and other optic neuropathies (Govindarajan et al., 2008; Huang et al., 2008; Das et al., 2013). Excessive $Ca^{2+}$ is also linked to and exacerbated by diminished mitochondrial motility (Rintoul and Reynolds, 2010; Liao et al., 2017). Interestingly, patients with ocular hypertension (elevated IOP) who do not develop optic neuropathy demonstrate a mitochondrial phenotype characterized by higher $Ca^{2+}$ buffering capabilities compared with mitochondria from control patients (Lascaratos et al., 2015).

The optic nerve's intrinsic attempts to restore functional levels of ATP are not without cost. During acute injury to the optic nerve, compensatory oxidative phosphorylation to boost mitochondrial ATP output leads to accelerated thinning of the RGC population (Zhu et al., 2020). This reduction is likely due to oxidative stress, which is broadly defined as imbalance between the production of reactive oxygen species (ROS) and the availability of antioxidants or radical scavengers to neutralize them. Reduced mitochondrial motility or increased pockets of stationary mitochondria increase local concentrations of $Ca^{2+}$, free radicals and ROS to potentially toxic level (Wallace, 1999; Lee et al., 2010; Rintoul and Reynolds, 2010; Liao et al., 2017). In turn, chronic elevations in ROS in glaucoma cause not only oxidative damage, but also impede ATP generation (Chrysostomou et al, 2013). Progression of visual field deficits in glaucoma patients is associated with reduced blood serum levels of the antioxidant enzyme superoxide dismutase (SOD), a major cellular defense against ROS accumulation (Li et al., 2020). Inflammation in glaucoma is likely to exacerbate oxidative stress in the optic nerve head and retina by inhibiting mitochondrial motility via nitric oxide (NO)-dependent signalling pathways (Trivli et al., 2019).

Evidence from the DBA/2J mouse directly links oxidative stress to progressive degeneration of the RGC projection to the brain. Age and IOP both increase retinal levels of markers for lipid peroxidation and other genes and proteins related to oxidative stress; dietary treatment with the anti-oxdidant α-lipoic acid ameliorates these effects and improves RGC survival (Inman et al., 2013). The coenzyme Q10 (CoQ10) helps maintain mitochondrial membrane potential, ATP synthesis and intracellular $Ca^{2+}$ homeostasis (Zhong et al., 2017); by inhibiting ROS formation, it also reduces oxidative stress in neurodegenerative conditions (Zhang et al., 2017). DBA/2J mice fed a dietary supplement of CoQ10 demonstrate increased retinal mitochondrial transcription factor A, which supports oxidative phosphorylation and ATP production, and reduced oxidative stress with improved axonal integrity and RGC survival (Lee et al., 2014). Interestingly, while α-lipoic acid increased astrocyte remodeling in the retina (Inman et al., 2013), CoQ10 reduced it in optic nerve head (Lee et al., 2014). It is worth noting that neither treatment completely abates progression in rodents. Additional evidence supporting a pathogenic role for oxidative stress in RGC degeneration in glaucoma is reviewed elsewhere (Almasieh et al., 2012).

Since dysregulation of $Ca^{2+}$ homeostasis and mitochondrial stress are intimately linked, it is curious that the unmyelinated RGC axon segment – which is more susceptible to both – remains as long as it does in glaucoma, outlasting both the myelinated segment and dendritic arbor (Calkins, 2012). The next section examines a phenomenon associated with early progression in an animal model that could shed light on this persistence.

## 3. Adaptive Responses to Stress

### 3.1. The Unmyelinated Axon Segment and Enhanced Excitability

Persistence of the unmyelinated axon segment in glaucoma is not simply happenstance but may reflect instead an important functional role in promoting an adaptive RGC response in glaucoma. As with other locations of high mitochondrial density in the axon (Barron et al., 2004), varicosities in the unmyelinated RGC axon segment also contain pockets of localized NaV subunits (Risner et al., 2018; 2020; Figure 3), which comprise multiple

types. The initiation and propagation of action potentials requires activation of the NaV1.6 voltage-gated sodium channel while the NaV1.2 subunit facilitates backpropagation of depolarization to the cell body and dendritic arbor (Hu et al., 2009; Spratt et al., 2019). In the retina, both subunits localize predominantly to RGC bodies and axons, consistent with their physiological role in neuronal excitation (Caldwell et al., 2000; Boico et al., 2003; Van Wart and Matthews, 2006; Van Hook et al. 2019).

Studies of progression in mice using microbead-induced elevations in IOP suggest that NaV subunits expressed in the RGC reorganize as part of an early adaptive role in glaucoma. We simplified and standardized microbead occlusion from earlier paradigms to provide an easy method to induce quick, sustainable, and moderate elevations in IOP in rodents (Sappington et al.,, 2010, Chen et al., 2011; Dapper et al., 2013; Calkins et al., 2018) and more recently in non-human primates (Lambert et al., 2019; 2020). In this model a small-volume injection of polystyrene microbeads into the anterior chamber impedes aqueous fluid outflow, causing an increase in IOP of about 35% that persists over several weeks. Mice demonstrate IOP-related deficits in spatial acuity concomitant with degradation of anterograde transport to the SC early, between one and two weeks (Risner et al., 2018; Cooper et al., 2020); axon degeneration in optic nerve follows deficits in axon transport (Ward et al., 2014; Weitlauf et al., 2014).

While distal axon function degrades early in microbead-induced elevations in IOP, at the proximal end of the axon other changes are taking place. Following two weeks of elevated IOP in mice, mitochondria-rich varicosities in the RGC unmyelinated axon segment enlarge and increase in density closer to the cell body (Risner et al., 2018). As they do, localization of NaV1.6 within the enlarged axonal varicosities increases, concomitant with enhanced excitability of multiple RGC types with increased spontaneous activity (Weitlauf et al., 2014; Risner et al., 2018; Tao et al., 2020). Excitability in this context includes increased depolarization of the resting membrane potential, which reduces the threshold for excitation. As a result, enhancement entails increased mean and integrated responses to light. Importantly, this increase occurs even for those cells demonstrating dendritic pruning (Figure 5). Enhancement is axogenic, dependent on NaV1.6 activation. Since axonal varicosities in the unmyelinated segment are rich in mitochondria (Wang et al., 2003), increased size likely accommodates more mitochondria to support extra NaV1.6 activity. Even so, both changes are transient, since by four weeks of elevation, levels of NaV1.6 diminish below control levels as does RGC excitability (Risner et al., 2018).

What could be the purpose of axogenic enhancement of RGC excitability? The answer may be linked to the physiological properties of the NaV1.6 subunit, which produces a persistent excitatory current that sustains higher rates of action potential generation compared to the NaV1.2 subunit (Rush et al., 2005). Even during brief periods of induced IOP elevation, certainly within the experimental window of enhanced excitability, some RGC dendritic arbors exhibit pruning with loss of conventional, excitatory glutamatergic synapses from bipolar cell axon terminals (Della Santina et al., 2013; El-Danaf et al., 2015; Ou et al., 2016; Risner et al., 2018). Increased NaV1.6 in the unmyelinated axon segment likely boosts the light response by facilitating excitation arising from the remaining intact dendritic arbor as a means to maintain signaling along the optic nerve to the brain. Two additional observations

support this idea. First, enhanced excitability also involves a depolarizing shift in the RGC resting membrane potential (Figure 5), which facilities NaV1.6's transition to an activated state (Hu et al., 2009), thereby necessitating less conventional depolarization from synapses in the dendritic arbor. Second, we also found that the compound action potential for mouse optic nerve during the period of enhanced excitability does not diminish (Cooper et al., 2020; McGrady et al., 2020). This means that whatever loss of excitation is occurring in the dendritic arbor during early progression does not translate to lost signaling in the nerve itself.

That NaV1.2 does not increase along with NaV1.6 likely protects already vulnerable RGC dendrites from excessive depolarization from backpropagation of the boosted axonal signal (Risner et al., 2020). In fact, during the period of enhancement, pruning appears to slow – many RGCs retain a full arbor and others demonstrate extensive dendritic remodeling (El-Danaf et al., 2015; Ou et al., 2016; Risner et al., 2018).The axogenic nature of enhanced excitability also reduces the chances of glutamate overload in the dendritic arbor, which could occur with an increase in conventional synaptic signaling. Excessive glutamate is associated with the transformation of mitochondria from elongated to round (Rintoul et al., 2003), which would exacerbate the challenge to mitochondrial transport within the unmyelinated axon segment (Rintoul and Reynolds, 2010).

### 3.2. Adaptation as a Catalyst for Degeneration

Enhanced excitability appears to be a general property that counters early neurodegenerative progression in glaucoma models. This phenomenon appears in both ganglion cells that respond to light onset or increments (ON cells) and those that respond to light offset or decrements (OFF cells), independent of the degree of dendritic pruning (Risner et al., 2018; Tao et al., 2020). This generality may help inform its purpose. From the standpoint of a single RGC, maintaining the capacity to generate action potentials and signal the brain in concert with neighbors must be a prerogative of survival in adulthood as it is in development. An increased rate of action potential firing improves correlated signaling between neurons (Barreiro and Ly, 2017); this may enable a struggling RGC to better encode information with retinotopic neighbors. Also, excitatory activity promotes Akt-dependent signaling and is necessary for maintaining synaptic strength, survival of post-synaptic neurons in central projection sites, and intact trophic signaling (Lu, 2003; Chiu et al., 2008; You et al., 2012; Rangaraju et al., 2014). In fact, Akt is one of a handful of protein kinases that can promote the neuroprotective influence of BDNF (Chen et al., 2013; Dong et al., 2018). In the SC of glaucomatous mice, both DBA/2J and microbead-induced, retinotopic regions of degraded RGC anterograde transport demonstrate focal upregulation of astrocyte-derived BDNF (Crish et al., 2013; Crish and Calkins, 2015). This injury-specific increase may explain the observed persistence of key synaptic structures in the SC and optic nerve even after anterograde transport is completely depleted and optic nerve degeneration has progressed considerably (Crish et al., 2010; Smith et al., 2016).

Apparently, whatever the adaptive benefit enhanced excitability has on maintaining RGC signaling, it is ultimately unsustainable, since the phenomenon is transient (Risner et al., 2018). After the period of enhancement, degradation of anterograde transport, visual

acuity, and axonal integrity in the optic nerve resumes and accelerates (Risner et al., 2018). Similarly, while mitochondrial oxidative phosphorylation increases retinal ATP immediately following optic nerve crush, the excess eventually promotes RGC degeneration (Zhu et al., 2020). Even though enhanced activity could help the individual RGC survive, from the standpoint of the population functioning in a *bioenergetic system*, dwindling metabolic resources combined with oxidative stress in the unmyelinated axon segment could simply tip the balance from pro-survival to pro-degenerative. Increased neuronal activity drives ATP synthesis, which depletes local glucose reserves (Brown et al., 2003; Rangaraju et al., 2014). Thus, those RGCs tapping a limited supply of ATP to support both NaV in the unmyelinated segment for enhanced excitability and active transport to distal axon segments are simply fighting a losing battle. Finally, while excitation could help an individual RGC maintain concerted signaling with the population, ultimately too much of a good thing is, well, not so good. As activity and the demand for ATP pushes the limit of mitochondrial capacity to buffer $Ca^{2+}$, the potential for excitotoxicity and oxidative damage increases (Arundine andTymianski, 2003). This could explain the beneficial effects of inhibition of $Ca^{2+}$ channels following acute optic nerve injury (Knöferle et al., 2010).

### 3.3. Astrocyte Remodeling Redistributes Resources

Neurons, including the RGC, do not normally store substantial levels of glucose-derived glycogen for use during periods of high metabolic demands, since too much glycogen can be neurotoxic (Bélanger et al., 2011; Rai et al., 2018). Rather, like astrocytes elsewhere in the brain (Gjedde et al., 2002), those that blanket the nerve fiber layer and distribute densely throughout the optic projection store glycogen that is converted to bioenergetic substrates (particularly lactate) that can be transferred to the RGC and its axon in conditions of increased activity, stress or injury (Brown et al., 2003; Brown and Ransom, 2015; Brown et al., 2019; Mori et al., 2020). While neurons have an intrinsic capacity to generate energy from glucose directly through glycolysis (Díaz-García et al., 2017), indirect transfer via astrocytes may be a more expedient route to supply focal needs for ATP along lengthy axon tracts. Next, we consider whether astrocytes too, like RGCs, respond dynamically to early progression.

Enhanced RGC excitability entails increased initiation and propagation of action potentials along the optic nerve for a given level of light stimulation, which places additional metabolic burden on the myelinated segment (Trevisiol et al., 2017). Accordingly, just days following microbead-induced elevations in IOP, glycogen levels in the hypertensive nerve drop below levels in the control nerve (Cooper et al., 2020; Figure 6A–B). This is consistent with the influence of elevated IOP on ATP content in the DBA/2J mouse optic nerve (Baltan et al., 2010). However, quite unexpectedly, in the brief period of RGC enhanced excitability that follows, glycogen levels in the stressed optic nerve increase above those of the control nerve. In turn, the control optic nerve demonstrates diminished glycogen below levels in naïve optic nerve and a corresponding decrease in the control compound action potential. Interestingly, by four weeks of elevation – at which point enhanced excitability has ceased (Risner et al., 2018) – glycogen content in control and hypertensive nerves equilibrated and dropped below naïve levels (Cooper et al., 2020).

The results from Cooper et al. (2020) indicate that the demand for energy exacted by enhanced excitability, along with myriad disease-relevant stressors, is met by an attempt by the optic projection to quickly distribute resources where needed, namely, to the hypertensive nerve. Importantly, the apparent transfer of metabolites occurs via an extensive, bilateral network of astrocytes formed by connexin 43-enriched gap junctions. When this protein is conditionally eliminated from astrocytes, the transfer of metabolites ceases, as does its compensatory effects to slow progression in the hypertensive optic projection. Finally, without intact gap junctions between astrocytes, anterograde transport to the SC and visual acuity degrade more rapidly with elevated IOP (Cooper et al., 2020). Similarly, in the DBA/2J optic nerve, diminished levels of connexin 43 is associated with more dramatic reductions in anterograde axon transport to the SC (Cooper et al., 2018).

Clearly, the idea of an astrocyte network in stasis unless oscillating between two extreme modes – quiescent or inflammatory – is far too simple. Other aspects of astrocyte organization in the optic nerve indicate that dynamic remodeling is an important aspect of progression. Injury or long-term exposure to disease-relevant stressors in the central nervous system results in replacement of neural tissue with an astrocytic scar, which impedes the possibility of systemic repair. Formation is preceded by a period of astrocyte reactivity, characterized by increased expression of intermediate filament proteins to support hypertrophy, inflammatory signaling, and proliferation or gliosis (Wang et al., 2018; reviewed in Lee et al., 2020). However, early progression in glaucoma involves a very different type of astrocyte remodeling (Figure 6C–E). In the optic nerve, prior to demonstrable degeneration of RGC axons, individual astrocyte processes undergo extensive motility within axon bundles that involves $Ca^{2+}$-dependent signaling (Lye-Barthel et al., 2013; Ho et al., 2014; Wang et al., 2017).

This motility appears to be linked to metabolic redistribution. In the DBA/2J mouse, prior to outright axon degeneration, several important morphological changes take place; these are described in Cooper et al. (2016; 2018). First, RGC axons in the myelinated optic nerve enlarge, demonstrating disordered axoplasm that corresponds to increased phosphorylation of neurofilaments. As axons expand, astrocyte processes withdraw from the extra-axonal milieu, retreating towards the perimeter of the nerve, causing the local density of mitochondria for a given cross-sectional area of optic nerve to decrease. This withdrawal likely corresponds to or even causes elongation of astrocyte processes in the longitudinal plane of the optic nerve in early progression (Lye-Barthel et al., 2013; Wang et al., 2017). In later stages as RGC axons degenerate, glial coverage of the DBA/2J optic nerve – primarily from hypertrophic astrocyte processes – increases proportionally (Bosco et al., 2016). Even as IOP increases with age in this model, the key determinant of astrocyte hypertrophy is axon survival (Figure 6E). Finally, remodeling of the astrocyte network is important for promoting RGC survival. Attenuating reorganization by conditional knockout of the signal transducer and activator of transcription 3 (STAT3) from astrocytes accelerates degeneration following optic nerve injury (Sun et al., 2017).

# 4. Discussion

## 4.1. Adaptive Responses for the RGC and Astrocytes

That the RGC is simply a bystander in glaucoma, passively withstanding the buffets of degenerative pressures, is simply not a tenable concept, given the physiological evidence that tells a far different story. For example, in the retina, even as dendritic pruning progresses for multiple RGC types exposed to elevated IOP, remodeling of thin distal processes actually can expand the arbor (El-Danaf and Huberman, 2015). Interestingly, following acute axon injury in the optic nerve, mTOR complex 2 signaling when activated by exogenous insulin can extend RGC dendrites (Agostinone et al., 2018). Dendrite extension with elevated IOP appears to coincide with the period of enhanced excitability, which amplifies the RGC response to light and pauses dendritic pruning with some evidence of field enlargement (Risner et al., 2018). Early adaptation also involves increased spontaneous RGC activity, which makes sense given that the resting membrane potential becomes more depolarized during this time (Weitlauf et al., 2014; Risner et al., 2018; Tao et al., 2020). These physiological changes are accompanied by increased expression of functionally specialized excitatory channels, both voltage-gated (Risner et al., 2018) and non-voltage gated (Weitlauf et al., 2014).

We do not understand what initiates enhanced RGC excitability, though mechanistically we know the phenomenon itself is axogenic, involving voltage-gated sodium channels (Risner et al., 2018). The changes summarized here (section 3.1) loosely fall under the rubric of “intrinsic” to the RGC itself – properties we can assess by probing the RGC directly. This intrinsic response is only part of the story of adaptation, the other arising from elements extrinsic to the RGC. As we have seen (section 3.3), astrocytes undergo significant remodeling, well before the hypertrophic reactivity that we associate with significant axon loss in the optic projection (Cooper et al., 2016; 2018). The withdrawal of astrocyte processes prior to degeneration in the DBA/2J nerve is accompanied by diminished mitochondrial density, which likely contributes to reduced ATP and compound action potential (Cooper et al., 2016; Baltan et al., 2010). A similar process may be at play for laterally oriented astrocytes in the glial lamina, which also withdraw from axon bundles (Li et al., 2015). In any case, the idea that astrocytes in glaucoma are limited in their response to transitioning from a quiescent state, generally supportive of RGC function, to a reactive state, both inflammatory and prodegenerative, is an oversimplification. As we will see below, throughout progression astrocytes respond to local cues in ways that have broader implications for the optic projection as a system.

## 4.2. Adaptation in Progression: Survival of the Fittest

The transfer of astrocytic metabolites from unstressed to stressed optic nerve in experimental glaucoma induces a commensurate decrease in the unstressed nerve’s capacity to adapt to glucose deprivation (Cooper et al., 2020; Figure 6A,B). In neural tissue, focal increases in activity induce a transition from oxidative metabolism to increased reliance on astrocyte-supplied lactate (Kasischke et al., 2004). Indeed boosting RGC expression of the monocarboxylate transporter 2, which shuttles lactate and other metabolites in and out of cells, reduces degeneration in both DBA2J and microbead glaucoma (Harun-Or-Rashid

et al., 2020). However, several critical functions of astrocytes are more energy-demanding than neurons; remodeling and reactivity ultimately may divert resources otherwise needed for survival of the optic projection (reviewed in Weber and Barros, 2015). As the transfer of metabolites from the unstressed to stressed retinal projection occurs during the period of enhanced excitability, the additional burden on astrocytes already taxed by remodeling and reactivity could ultimately cause widespread failure of the bioenergetic network, thereby increased metabolic and oxidative stress in the system. A similar cascade may contribute to pathogenesis in Alzheimer's disease, in which astrocyte reactivity essentially syphons limited metabolic resources away from neurons (Steele and Robinson, 2012). The same reservoir of mitochondria that supports the metabolic needs of astrocytes also renders them susceptible to oxidative stress (Noh et al., 2013).

Astrocyte glia in the optic nerve respond to glutamate and potassium ($K^+$) extruded by axons at nodes of Ranvier with $Ca^{2+}$-dependent ATP release that stimulates glycogen turnover and inter-astrocyte communication within the gap-junction coupled network (reviewed in Butt et al., 2004; Kasischke et al., 2004). In effect, astrocytes receive a copy of the RGC signal as an index of activity that in turn can modulate availability of metabolites useful for the axon (Ransom and Orkand, 1996; Magistretti et al., 1999). As well, RGC axons also form intermittent connections to neighbors via gap junctions (Smedowski et al., 2020). These could serve to smooth focal activity, thereby reducing noise in the signal copied to astrocytes within a local network. It seems reasonable then that RGC enhanced excitability early in progression could serve as a catalyst to redirect resources through the astrocyte network to impede progression (Figure 7). This apparent redirection is extensive, from one optic projection to the other (Cooper et al., 2020; section 3.3). There is precedence for large-scale signaling. In optic nerve trauma, activation of the ATP-gated purinergic P2X7 receptor influences inflammation in the contralateral projection (Nadal-Nicolás et al., 2016).

Conversely, bundles of axons struggling to maintain concerted firing early in progression could evoke the opposite response, eliciting the redirection of resources that causes rather than reflects enhanced excitability. A corollary of this hypothesis is that as astrocytes monitor axonal depolarization within an individual optic nerve, focal redirection could deprive specific bundles of axons necessary metabolites, should their signaling become erratic. Remodeling of the gap-junction coupled network could serve to define units of functional vs. dysfunctional axons (Weber and Barros, 2015). In the DBA/2J optic nerve, the high degree of pre-degenerative astrocyte remodeling we observed includes withdrawal from bundles of axons that have accumulated phosphorylated neurofilaments (Cooper et al., 2016; 2018; see also Li et al., 2015). Such a response could indicate a thresholding trigger, in which astrocytes monitoring axon health redirect limited supplies of metabolites to better functioning axon modules to maximize survival of the fittest throughout the optic projection.

### 4.3. Revisiting the Nerve Head in Progression

For the adaptive responses described (Figure 7), the unmyelinated segment is critical, as the likely locus of enhanced excitability (section 3.1). At the optic nerve head, near the junction between the unmyelinated and myelinated segments, failed transport within RGC axons subjected to glaucomatous stressors appears to cause accumulation of important

intraaxonal cargo, such as amyloid precursor protein (Chidlow et al., 2011; Korneva et al., 2020; Maddineni et al., 2020), and reflects important cytoskeletal changes in axons that may influence functional transport (Balaratnasingam et al., 2007). Interestingly, a change in the gradient between IOP and cerebral spinal fluid pressure rather than absolute increases in pressure increases mitochondrial COX in the laminar region of the nerve head in pigs (Balaratnasingam et al., 2009). Thus, there is no doubt that this transition site demarcates physiological differences in how transport proceeds between the two segments. In this zone as well, astrocytes play a critical phagocytic role in clearing axonal debris, possibly from slowed transport of organelles (Nguyen et al., 2011). One idea is that RGC axons die back from or undergo axotomy at this juncture due to IOP-related insult (Soto et al., 2008; Li et al., 2015). Higher elevations in IOP that most certainly involve ischemic insult could lead to just such a scenario, along with rapid dendritic pruning in the retina and secondary loss of other retinal neurons through retrograde degeneration, akin to nerve crush or transection. However, progressive glaucoma typically does not involve such extreme IOP elevation (AGIS Investigators, 2000). Since some functional recovery is possible in patients undergoing treatment to lower IOP (Venture and Porciatti, 2005; Musch et al., 2014), dramatic mechanical separation of axonal segments is likely a special property of acute experimental models.

The nerve head is a hotbed for disease-relevant stressors that influence axon function and structure (section 1.2); progression from this point travels in both the anterograde and retrograde directions (Figure 2). Given the biomechanical strain associated with optic nerve head remodeling (Sigal and Ethier, 2009; Burgoyne et al., 2011; Girard et al., 2011; Quigley, 2015), one must consider whether spatially expansive signaling could arise from such strain and influence progression. An obvious candidate is ATP, working through purinergic receptors localized to RGC axons. Mechanical stimulation of many tissues elicits robust release of ATP, including conditions that incite inflammation (Mikolajewicz et al., 2018; 2019). Human optic nerve astrocytes exposed to mechanical stress similarly release ATP through pannexin channels (Beckel et al., 2014). In the retina, the RGC expresses the ATP-gated purinergic P2X7 receptor, the deletion of which delays degeneration following axotomy (Mitchell et al., 2008; Nadal-Nicolás et al., 2016). It seems reasonable that astrocyte remodeling due to glaucomatous strain, coupled with increased axonal demand for energy, could elicit changes in local ATP concentration. Since P2X7 receptors activate and gate influx of cations like $Na^+$ and $Ca^{2+}$ with very high concentrations of ATP (millimolar levels; Illes, 2020), astrocyte ATP could serve two purposes. Low concentrations of ATP extruded by astrocytes could increase glycogen turnover and inter-astrocyte communication (Butt et al., 2004; Kasischke et al., 2004), as physiological need demands, while higher concentrations would elicit cation dysregulation and $Ca^{2+}$-dependent cytoskeletal degradation. In this way, increased bioenergic stress within the system transforms the protective nature of metabolic redistribution into one that ultimately accelerates system failure and wide-spread degeneration. The key to new protective therapies then could lie within modulating the delicate balance of astrocyte-axon interactions.

### 4.4. Inflammation – not a Third Wheel

Pathogenesis in glaucoma, which is taken here to indicate the neurodegenerative processes that lead to vision loss, is not just about metabolic and oxidative stress, which are closely related. Glaucoma also involves diverse inflammatory components spanning anterior segment to central optic projection sites in the brain; these are reviewed amply elsewhere and so are not included here (Baudouin et al., 2020; Wareham et al., 2020). Even so, it is remiss to believe that oxidative and metabolic stress are easily dissociated from inflammatory contributors to progression. They are not. Just as metabolic and oxidative stress are linked, inflammation and oxidative stress go hand in hand, as many critical intracellular and extracellular signaling components create a substrate for crosstalk between them. For example, just as ROS in neurons can activate pro-inflammatory transcription factors that induce local immune cells to secrete signals that amplify the immune response, immune cells can enhance ROS production that leads to oxidative stress (Herman et al., 2018). As we have seen, dysregulation of axonal $Ca^{2+}$ contributes to oxidative stress through diminished mitochondrial motility (Rintoul and Reynolds, 2010; Liao et al., 2017). In a rat model of acute optic neuritis, an inhibitor of the $Ca^{2+}$-activated protease calpain decreased inflammatory mediators, including cytokines, along with microglial and astrocyte reactivity (Das et al., 2013).

In the context of neurodegeneration, the term “inflammation” itself has evolved greatly and now is invoked – rightly or wrongly – in relation to a multitude of processes involving glial activity. These range from how we traditionally think of inflammation in terms of activation and recruitment of immune cells to remodeling of glial cytoskeletal components to neurovascular coupling and vascular permeability to dendritic pruning and synapse elimination. Indeed, the early remodeling of astrocyte process in the optic nerve we describe for DBA/2J mice could be considered a new facet of glial reactivity and therefore an aspect of inflammation (Figure 6; Cooper et al., 2016; 2018). As noted above (section 4.3), astrocyte remodeling likely modulates local concentrations of ATP, activating $Ca^{2+}$-dependent cascades via P2X7 receptors localized to RGCs or their axons. However, microglia also express P2X7 receptors, activation of which triggers the release of inflammatory cytokines and ROS (Illes, 2020). In this case, we see that metabolic signaling and inflammation are linked.

Primary inflammatory diseases of the central nervous system such as multiple sclerosis are characterized by strong immune-mediated responses that elicit neurodegeneration (Degan et al., 2018). The neurodegenerative events described here and the adaptive responses that appear to counter them (Figure 7) involve combinations of glial reactivity, activation of complement-mediated pathways (e.g., synapse elimination), immune cell recruitment, and the production and secretion of inflammation mediators including cytokines and chemokines (Baudouin et al., 2020). In most types of glaucoma, but not all (Rieck, 2013), these responses are secondary to the primary injury to RGCs elicited by sensitivity to IOP. Astrocytes as well as microglia are key regulators of innate and adaptive immune responses (Colombo and Farina, 2016). Since metabolic redistribution and very likely enhanced excitability so intimately involve astrocyte remodeling, it is possible that they too elicit other components of neuroinflammation, including both pro-degenerative and reparative elements.

### 4.5. Relevance to Glaucoma Patients

Metabolic redistribution and enhanced excitability each represent a form of adaptative remodeling. Enhanced excitability involves reorganization of excitatory signaling substrates at the level of individual axons, while metabolic redistribution involves the movement of bioenergetic resources through astrocyte networks at the level of the optic projection. Each in different ways appears to counter progression, however transiently, by supporting RGC signaling along the optic nerve (Risner et al., 2018; Cooper et al., 2020). Given this protective element, it is reasonable to ask whether adaptive remodeling could be harnessed therapeutically for human patients.

As we have seen (section 4.2), the transient nature of adaptive remodeling may reflect limited bioenergetic resources for a system already taxed by primary pathogenic features of progression. There is evidence from animal studies that boosting such resources slows progression. In the DBA/2J mouse, levels of nicotinamide adenine dinucleotide ($NAD^+$), a cofactor central to all cellular metabolism, decreases with age (Williams et al., 2017). Both oral administration of the $NAD^+$ precursor nicotinamide (vitamin $B_3$) and directed gene upregulation of *Nmnat1*, a key $NAD^+$-producing enzyme, protects RGCs and slows progression was protective (Williams et al., 2017). Along these lines, oral supplementation with pyruvate, a product of glycolysis, is neuroprotective in both the DBA/2J mouse and in an inducible rat model of glaucoma, reducing both anterograde transport deficits and axon degeneration in the optic nerve (Harder et al., 2020).

These results may show a relatively simple path forward to slowing neurodegeneration in glaucoma patients, by providing additional metabolic resources to the optic projection. On the other hand, neuroprotective regimens that work in rodents do not necessarily translate to models involving larger mammals, including non-human primates, let alone to human patients. For example, inhibitors of p38 MAP kinases that attempt to reduce inflammatory signaling reduce degeneration in rats and mice but are far less effective in monkeys (Lambert et al., 2020). It is enticing to postulate that interventions involving bioenergetics are effective because they feed and extend adaptive remodeling. If so, a better way forward in glaucoma may identify targets within metabolic redistribution and enhanced excitability that enable their protective capacities while balancing oxidative byproducts that result from the use of additional fuel sources. The key lies in understanding collectively the adaptive pressures facing RGCs and astrocytes as local neuroexcitatory units that must respond to the overriding needs of the entire optic projection.

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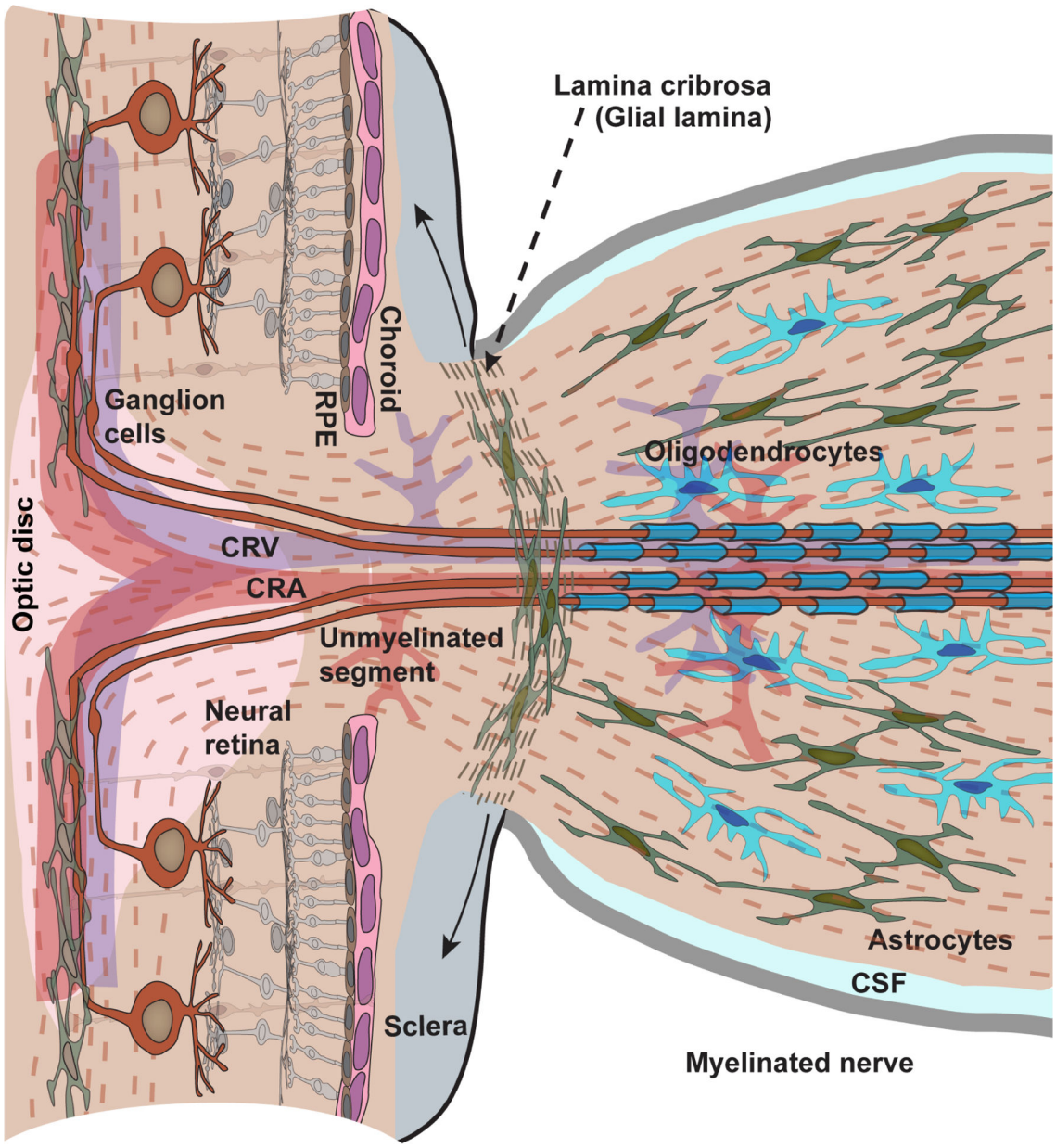


**Figure 1. Basic structure of the optic nerve head.**

The vertebrate retina is inverted with respect to the path of light; the photoreceptor-renewing retinal pigment epithelium (RPE) and choroid are most proximal to the sclera. Retinal ganglion cell axons are unmyelinated in the retina and in entering the nerve head through to the prelaminar region. In passing through the lamina cribrosa, axons become myelinated by oligodendrocytes, first in the retrolaminar region and then throughout the optic nerve, which is bathed in cerebrospinal fluid (CSF). The cribrosa is a critical site of biomechanical coupling to ocular stress through attachments with the peripapillary region of the sclera (solid arrows); its normal backward bowing becomes dramatically more pronounced in glaucoma. In place of a well-defined cribrosa and retrolaminar region the rodent nerve head demonstrates a glial lamina of densely distributed astrocytes and a myelination transition

zone, respectively. Astrocyte glia also interact with RGC axons in the retina and in the myelinated segment of the optic nerve. The central retina artery (CRA) and vein (CRV) enter the retina through the optic disc.

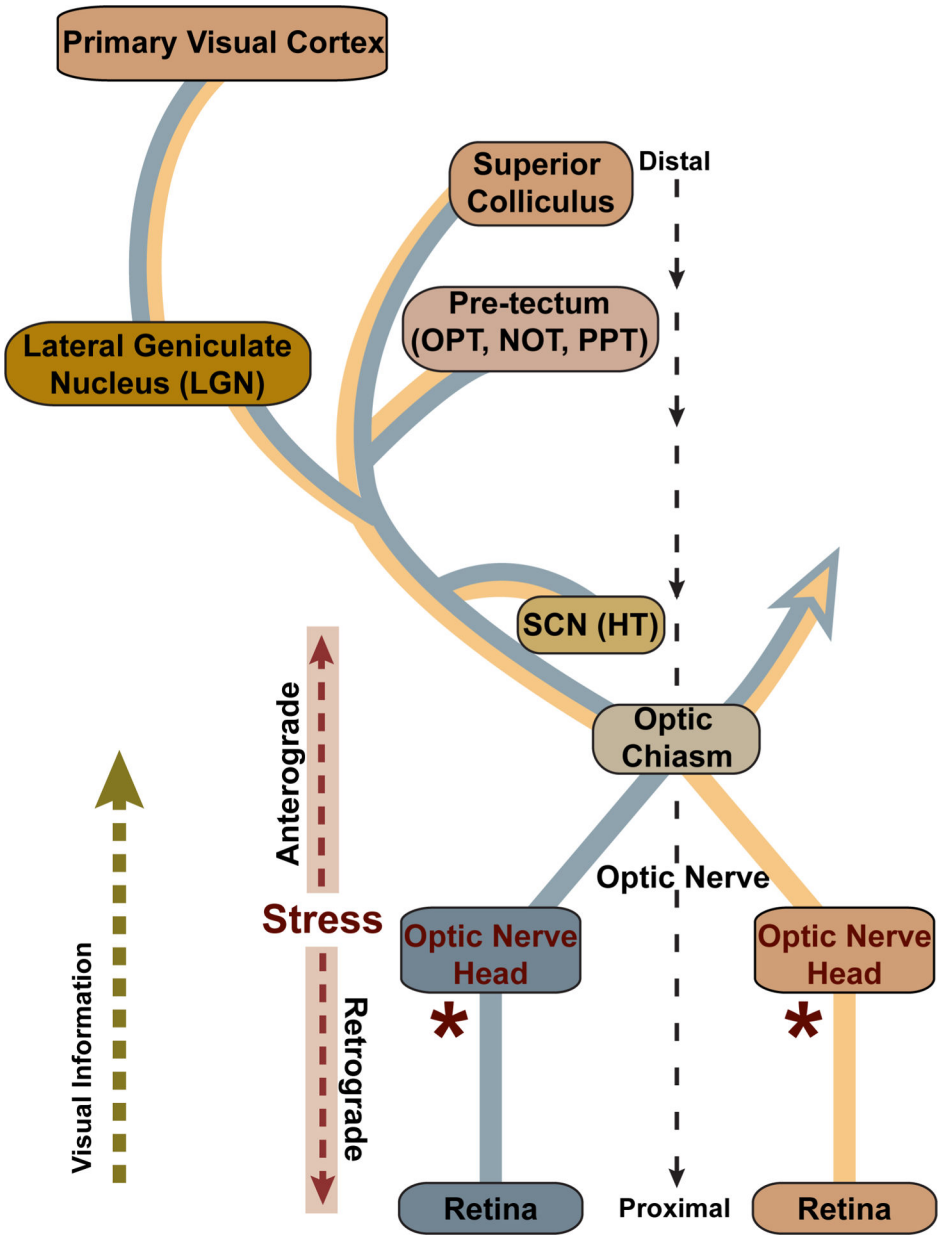


**Figure 2. Visual projection in glaucoma.**

Retinal ganglion cell axons project through the optic nerve head and at the optic chiasm join either the ipsilateral or contralateral optic tract to central brain targets. The strength of each projection varies by species; the rodent retina projects primarily contralaterally, while the primate retina projects equally to both. Central targets include the suprachiasmatic nucleus (SCN) of the hypothalamus (HT) and the olivary pretectal nucleus (OPT), nucleus of the optic tract (NOT), and posterior pretectal (PPT) nucleus of the pretectum. The lateral geniculate nucleus (LGN) of the thalamus is the primary RGC recipient in primates, while in rodents all or nearly all ganglion cells project to the superior colliculus (SC), while extending axon collaterals to nuclei more proximal to the retina. In all mammals the SC is the most distal direct target, while the LGN provides all direct projection to the primary

visual cortex. In the glaucoma, stress at the optic nerve head (*) is conveyed along the ganglion cell axon both towards the brain, in the anterograde direction, and towards the retina, in the retrograde direction. Modified from Crish and Calkins (2015).

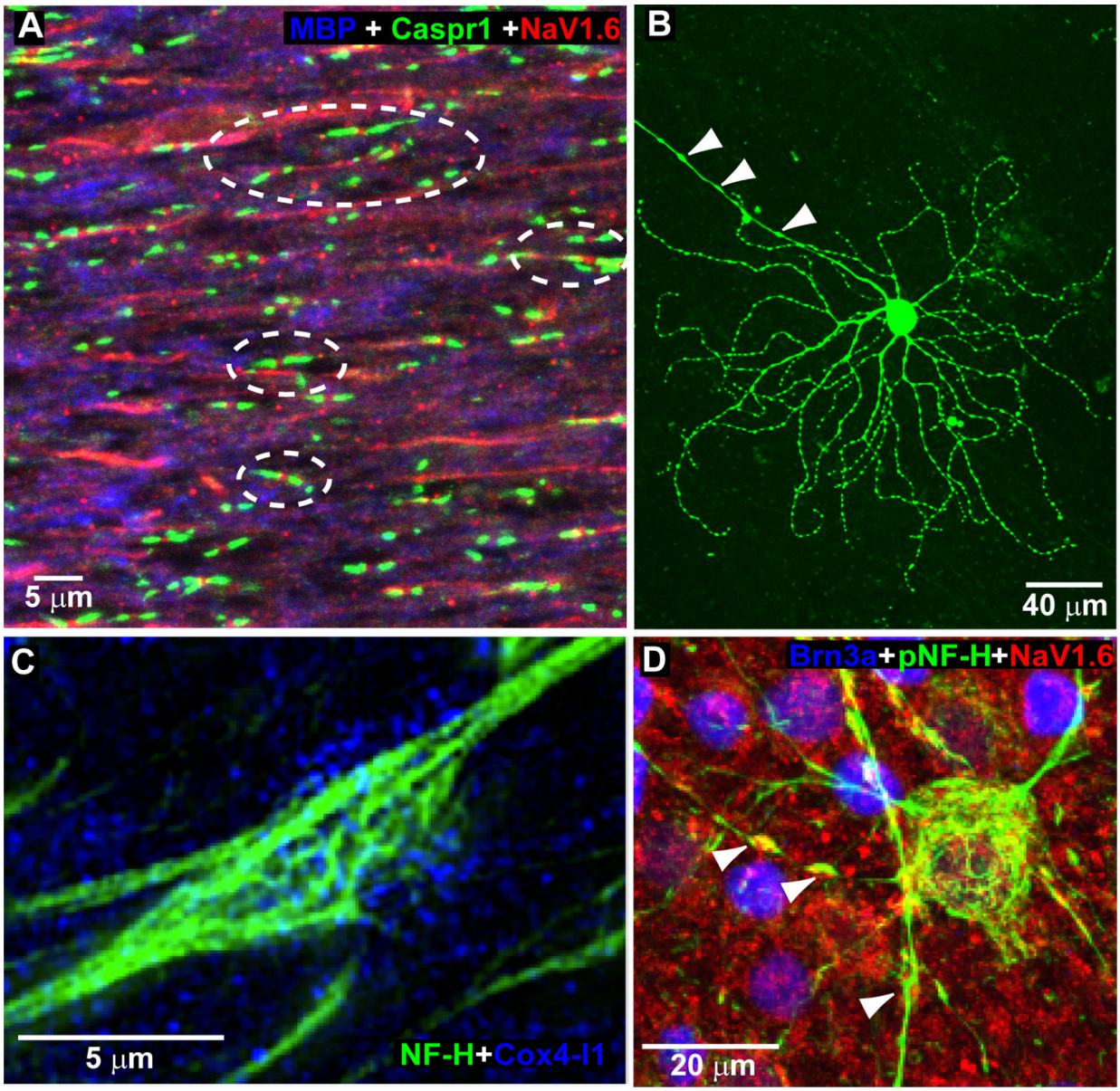


**Figure 3. RGC axonal ultrastructure.**

**A.** Mouse optic nerve labeled with antibodies against myelin basic protein (MBP) showing paranodal contactin-associated protein 1 (Caspr1) flanking nodes of Ranvier containing the voltage-gated sodium channel NaV1.6 subunit. **B.** Mouse ganglion cell in wholemount preparation following intracellular dye filling to illuminate dendritic arbor and unmyelinated axon segment rich in varicosities (arrowheads). **C.** High magnification micrograph of RGC axonal varicosity labeled for neurofilament heavy chains (NF-H) contains mitochondria rich in the enzyme cytochrome c oxidase subunit 4 isoform 1 (Cox4-I1). **D.** Phosphorylated neurofilament heavy chains (pNF-H) in Brn3a-labeled ganglion cell highlights varicosities in the unmyelinated axon segment containing NaV1.6 (arrowheads).

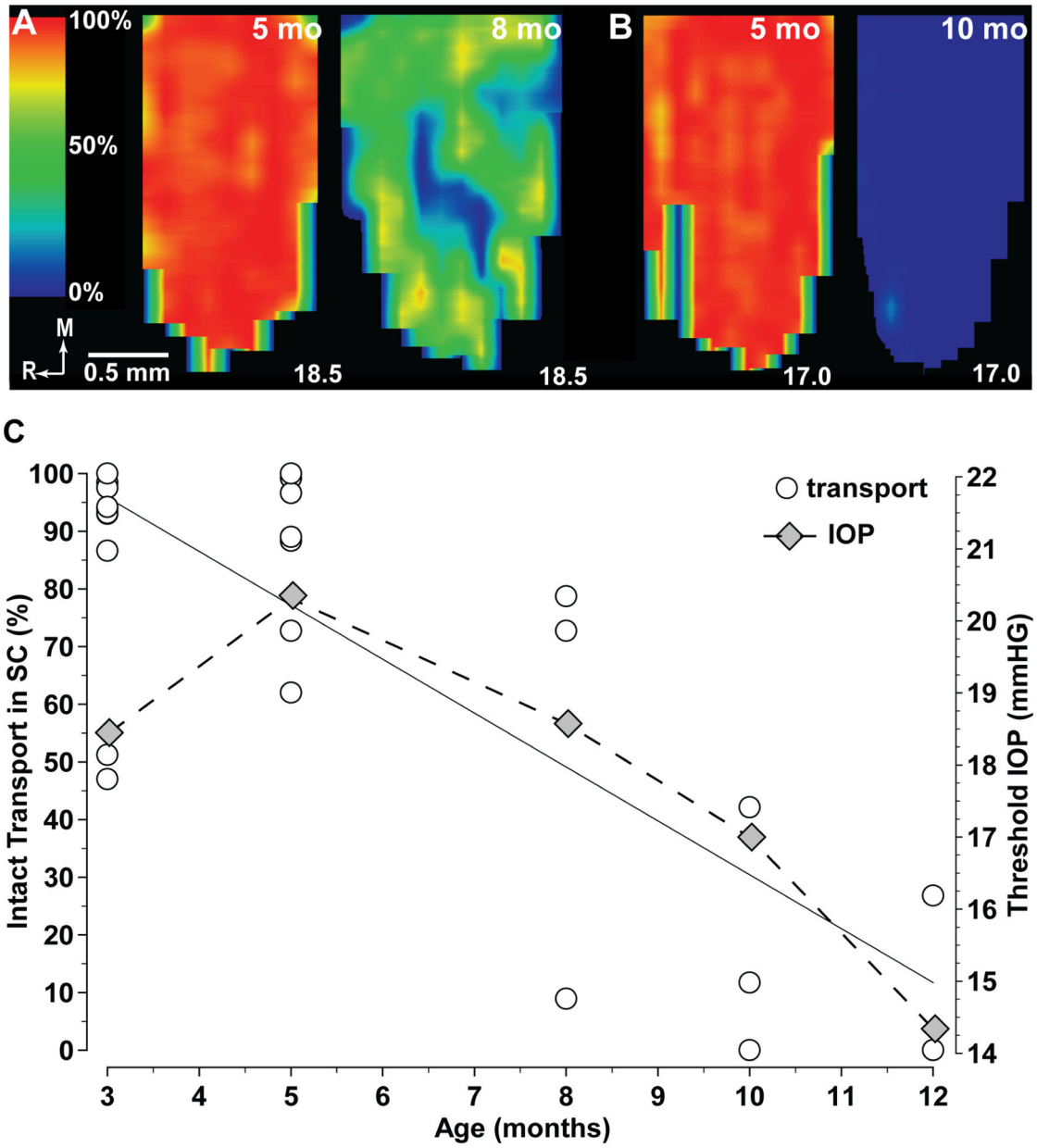


**Figure 4. Age is predominating determinate of anterograde axonal transport.**

**A.** Retinotopic maps of superior colliculus (SC) from five-month (left) and eight-month (right) DBA/2J mice, each receiving retinal projections from eyes with mean lifetime IOP of 18.5 mm Hg. Maps show levels of cholera toxin B transported from the retina varying from 100% (red) to 50% (green) to 0% (blue). **B.** Maps of five-month (left) and 10-month (right) DBA/2J SC, with corresponding mean IOP of 17.0 mm Hg. **C.** Intact anterograde transport of cholera toxin b to the DBA/2J SC expressed as the fraction of the retinotopic map with ≥ 70% signal density vs. age in months (top). All SC received retinal projection from eyes with mean lifetime IOP < 20 mm Hg. Best-fitting regression shows a significant correlation ($r^2$=0.64; p<0.001). Threshold mean lifetime IOP to induce a 50% loss of intact anterograde transport to the DBA/2J SC diminishes with age (bottom).

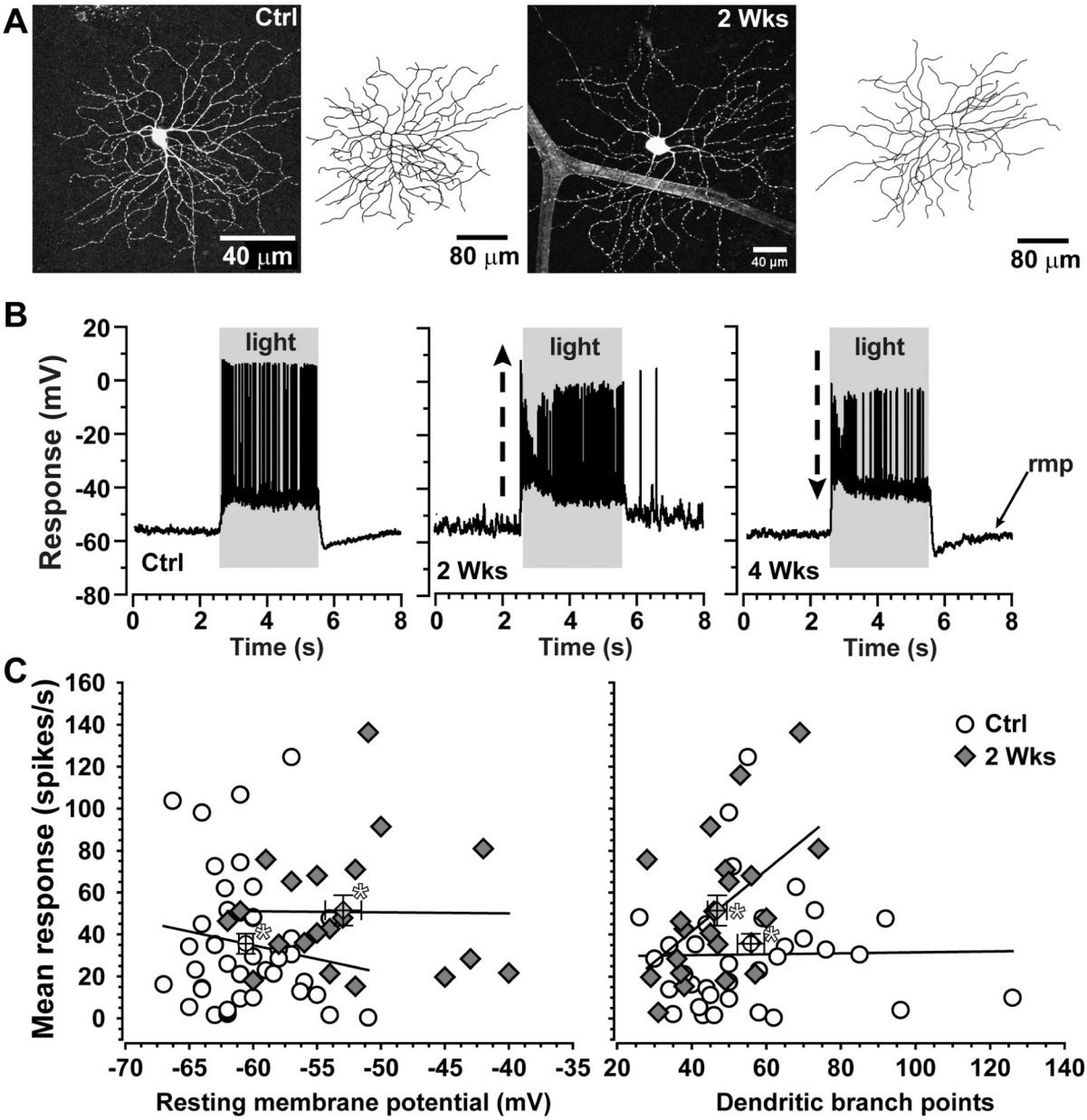


**Figure 5. Enhanced excitability is independent of dendritic pruning.**

**A.** Dendritic arbor of an ON-Sustained RGC from control (left) mouse retina following intracellular filling during physiological recording. Same cell type following two weeks of microbead-induced IOP elevation (+35%) shows loss of dendritic branch points (right). **B.** Current-clamp recordings from single ON-Sustained RGCs from control mouse retina (left) and from retinas following two (middle) and four (right) weeks of elevated IOP. At two weeks, the RGC demonstrates enhanced response to light and a more depolarized resting membrane potential (rmp); by four weeks, response diminishes compared to control. **C.** Mean response to light after two weeks of elevated IOP increases as resting membrane potential becomes more depolarized (left) and dendritic branch points diminish (right) compared to control (* marks averages ± SEM). Despite having fewer branch points overall,

the mean response of ON-Sustained RGCs at two weeks increases with dendritic complexity ($p=0.01$). Experiments described in Risner et al. (2018).

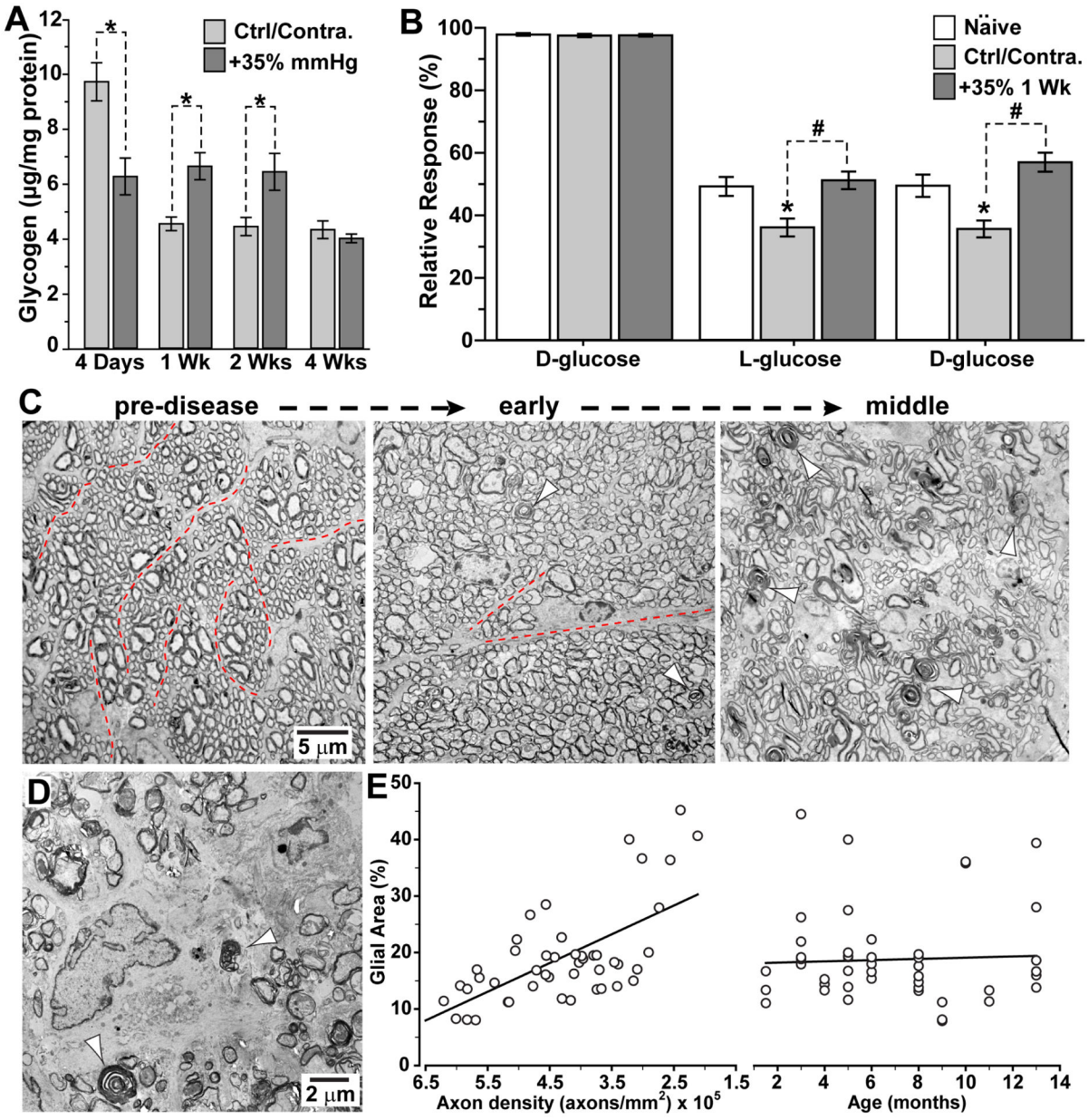


**Figure 6. Astrocyte metabolism and remodeling in the optic nerve.**

**A.** In C57 mice, microbead-induced elevations in IOP (+35%) initially diminish astrocyte-stored glycogen in the optic nerve compared to the control/contralateral control nerve; after one and two weeks, glycogen in the stressed nerve increases at the expense of the control nerve (*, $p \leq 0.05$). By four weeks, glycogen equilibrates between the nerves. **B.** As a result of glycogen imbalance, compound action potential of C57 optic nerve following one week of elevated IOP responds stronger during and recovers faster from D-glucose deprivation than control/contralateral control nerve (#, $p<0.001$); control nerve also response more weakly than naïve nerve (*, $p \leq 0.002$). **C.** Electron micrographs of cross-sections through DBA/2J optic nerve demonstrate intact axon fascicles bound by thin astrocyte processes (dotted lines) prior to progression (left). In the early stages, axons enlarge, and

astrocyte processes withdraw with sparse examples of degenerating profiles (arrowheads). As progression continues (right), fascicles become disorganized as degeneration accelerates. **D.** In final stages, DBA/2J nerve demonstrates abundant degenerating profiles (arrowheads) as astrocyte hypertrophy predominates over intact axons. **E.** The density of intact RGC axons determines the extent of astrocyte hypertrophy in the DBA/2J more so than age ($r^2$=0.42; p<0.001). A, B: modified from Cooper et al. (2020). C,D: see also Cooper et al., 2016; 2018.

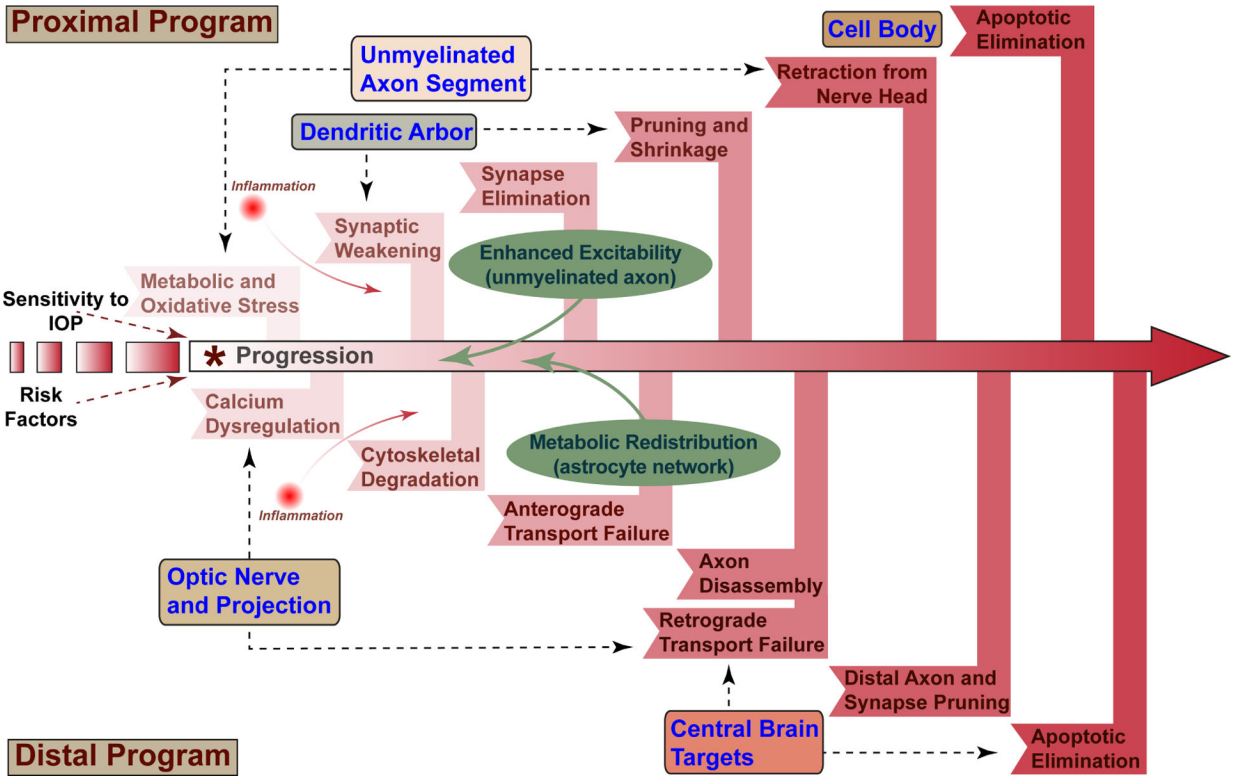


**Figure 7. Progression involves adaptive responses slow progression.**

Sensitivity to IOP and other risk factors combine to increase susceptibility to glaucomatous neurodegeneration through stress conveyed to the RGC axon within the optic nerve head (*). Progression comprises a proximal program influencing the unmyelinated axon segment, RGC dendritic arbor, and cell body and a distal program affecting the myelinated axon and its projection into central brain targets. Each program includes inflammatory signaling, which contributes in multiple ways to pathogenesis. In this model, early metabolic and oxidative stress originating in the unmyelinated axon segment leads to both calcium dysregulation and axonopathy in the optic nerve and synaptic and dendritic pruning in the retina. Enhanced excitability originating in the unmyelinated segment and astrocyte-mediated metabolic redistribution represent adaptive responses that impede proximal and distal programs, respectively.