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A Biomechanical Paradigm for Axonal Insult Within the Optic Nerve Head

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Abstract

Rosario Hernandez—This article is dedicated to Rosario Hernandez for her warm support of my own work and her genuine enthusiasm for the work of her colleagues throughout her career. I first met Rosario as a research fellow in Harry Quigley's laboratory between 1991 and 1993. Along with Harry, John Morrison, Elaine Johnson, Abe Clark, Colm O'Brien and many others, Rosario's work has provided lamina cribrosa astrocyte cellular mechanisms that are biomechanically plausible and in so doing provided credibility to early notions of the optic nerve head (ONH) as a biomechanical structure.

We owe a large intellectual debt to Rosario for her dogged persistence in the characterization of the ONH astrocyte and lamina cribrosacyte in age and disease. Two questions run through her work and remain of central importance today. First, how do astrocytes respond to and alter the biomechanical environment of the ONH and the physiologic stresses created therein? Second, how do these physiologic demands on the astrocyte influence their ability to deliver the support to retinal ganglion cell axon transport and flow against the translaminar pressure gradient?

The purpose of this article is to summarize what is known about the biomechanical determinants of retinal ganglion cell axon physiology within the ONH in the optic neuropathy of aging and Glaucoma. My goal is to provide a biomechanical framework for this discussion. This framework assumes that the ONH astrocytes and glia fundamentally support and influence both the lamina cribrosa extracellular matrix and retinal ganglion cell axon physiology. Rosario Hernandez was one of the first investigators to recognize the implications of this unique circumstance. Many of the ideas contained herein have been initially presented within or derived from her work (Hernandez, M.R., 2000. The optic nerve head in glaucoma: role of astrocytes in tissue remodeling. Prog Retin Eye Res. 19, 297–321.; Hernandez, M.R., Pena, J.D., 1997. The optic nerve head in glaucomatous optic neuropathy. Arch Ophthalmol. 115, 389–395.).

Keywords

Glaucoma; Acute IOP elevation; Optic Nerve Head; Neural Canal; Lamina Cribrosa Position and Thickness; Peripapillary Scleral Position and Thickness; Post-NCO Total Prelaminar Volume

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Glaucoma, Cupping and Axonal Insult Within the Optic Nerve Head (ONH)

While glaucomatous damage to the visual system likely includes important pathophysiologies within the retinal ganglion cell body (Asai et al., 1987; Garcia-Valenzuela et al., 1995; Quigley, 1995a; Quigley et al., 2000; Quigley et al., 1995; Weber et al., 1998) photoreceptors (Janssen et al., 1996; Kendell et al., 1995; Nork et al., 2000; Panda and Jonas, 1992; Wygnanski et al., 1995), lateral geniculate body (Yucel et al., 2000; Yucel et al., 2001, 2003) and visual cortex (Yucel et al., 2003), strong evidence suggests that damage to the retinal ganglion cell axons within the lamina cribrosa of the ONH (Bellezza et al., 2003b; Burgoyne et al., 2004; Gaasterland et al., 1978; Minckler et al., 1977; Quigley et al., 1981; Quigley and Green, 1979) is a central pathophysiology. Recent studies in the monkey (Bellezza et al., 2003b; Burgoyne et al., 2004; Downs et al., 2005; Downs et al., 2007; Yang et al., 2007a; Yang et al., 2007b), rat (Cepurna et al., 2005; Johnson et al., 2000; Johnson et al., 1996), and mouse (Danias et al., 2003; Filippopoulos et al., 2006; Howell et al., 2007; Jakobs et al., 2005; Schlamp et al., 2006) support the importance of the ONH, by describing profound alterations and axonal transport disruption within the prelaminar, laminar and retrolaminar tissues of the ONH at the earliest detectable stage of experimental glaucoma.

The ONH tissues make up a dynamic environment wherein 1.2 to 2.0 million RGC axons converge, turn, and exit the eye through the inner (Bruch's Membrane opening) and outer (scleral) portions of the neural canal (Figure 1). Within the scleral portion of the canal, the bundled axons pass, through a 3-dimensional (3D) meshwork of astrocyte-covered, capillary containing, connective tissue beams known as the lamina cribrosa (Figure 1). Within the lamina, axonal nutrition is thought to depend upon the movement of oxygen and nutrients from the laminar capillaries, through the laminar beam extracellular matrix, across the astrocyte basement membrane into the astrocyte, finally reaching the peripheral and central axons of each bundle, via cell processes (Anderson, 1969).

"Cupping" is a clinical term which is used to describe ONH structural change in all forms of optic neuropathy (Bianchi-Marzoli et al., 1995; Greenfield et al., 1998; Johns et al., 1989; Klein et al., 2006; Pederson and Anderson, 1980; Pederson and Gaasterland, 1984; Schwartz et al., 1975; Sponsel et al., 2001). However, "cupping" is also used as a synonym for the pathophysiology of glaucomatous damage to the ONH (Anderson and Cynader, 1997; Kalvin et al., 1966; Quigley and Green, 1979; Vrabec, 1976). Because the clinical and pathophysiologic contexts for "cupping" are seldom clarified there is a confusing literature regarding the presence, importance and meaning of "cupping" in a variety of disorders (Alward, 2004; Ambati and Rizzo, 2001; Bianchi-Marzoli et al., 1995; Danesh-Meyer et al., 2001; Greenfield, 1999; Greenfield et al., 1998; Hall et al., 2006; Hayreh and Jonas, 2001; Johns et al., 1989; Klein et al., 2006; Manor, 1995; Nicolela and Drance, 1996; Nicolela et al., 2003; Orgul et al., 1994; Pederson and Anderson, 1980; Pederson and Gaasterland, 1984; Piette and Sergott, 2006; Quigley and Anderson, 1977; Schwartz et al., 1975; Sharma et al., 1999; Sonty and Schwartz, 1983; Sponsel et al., 2001; Trobe et al., 1980; Votruba et al., 2003). Cupping in glaucoma is highly variable (Nicolela and Drance, 1996; Nicolela et al., 2003). We have previously proposed that only "laminar" or "deep" forms of "cupping" (those that include a connective tissue component) are pathognomonic for glaucoma (Burgoyne and Downs, 2008; Burgoyne et al., 2005; Yang et al., 2007a). We now further clarify that even a glaucomatous form of "cupping" is only one manifestation of the underlying pathophysiologic processes which drive the optic neuropathy of glaucoma. Cupping is therefore a manifestation of the neuropathy of glaucoma, not the optic neuropathy itself.

Damage to the retinal ganglion cell axon within the ONH is a second component of the optic neuropathy of glaucoma, but it also is not the optic neuropathy itself. While the

pathophysiology of RGC axon damage is of fundamental importance in preserving vision, it may be only one component of, (or secondary to) the larger pathophysiologic events that drive the neuropathy. ONH biomechanics seeks to identify and explain the larger intraocular pressure (IOP)-related and non IOP-related determinants of glaucomatous damage so as to build a clinical science to predict eye-specific ONH behavior and susceptibility to a given level of IOP (Burgoyne and Downs, 2008; Burgoyne et al., 2005; Burgoyne and Morrison, 2001; Downs et al., 2009; Sigal and Ethier, 2009a; Sigal and Ethier, 2009b; Sigal et al., 2009a, b; Sigal et al., 2010b).

The Biomechanical Paradigm of Glaucomatous damage to the ONH

The biomechanical paradigm of glaucomatous ONH damage does not argue that the ONH is the earliest or only site of damage. ONH biomechanics provides a framework for explaining how IOP-related stress (force/cross-sectional area of the tissue experiencing that force) and strain (a measure of local deformation of a tissue induced by applied stress) within the load-bearing tissues of the ONH influence the physiology and pathophysiology of all three ONH tissue types. These include: 1) the connective tissues (load-bearing connective tissues of the peripapillary sclera, scleral canal wall, and lamina cribrosa), 2) the neural tissues (retinal ganglion cell axons), and 3) the cells which exist alone or in contact with both 1 and 2 (astrocytes, glial cells, endothelial cells, and pericytes and their basement membranes) (Burgoyne and Downs, 2008; Burgoyne et al., 2005; Burgoyne and Morrison, 2001; Downs et al., 2009; Sigal and Ethier, 2009a; Sigal and Ethier, 2009b; Sigal et al., 2009a, b; Sigal et al., 2010b).

ONH biomechanics provides a logic by which non-IOP-related risk factors such as ischemia, inflammation, auto-immunity, astrocyte and glial molecular biology are influenced by or interact with the effects of IOP. ONH biomechanics attempts to combine these] *"non IOP-related"* factors with laminar and peripapillary scleral connective tissue geometry and material properties (strength, stiffness, structural rigidity, compliance and nutrient diffusion properties) to explain the physiology of normal ONH aging, ONH susceptibility to IOP, and the clinical manifestation of all forms of optic neuropathy. The engineering components of ONH biomechanics have been the focus of a series of recent reports (Burgoyne and Downs, 2008; Burgoyne et al., 2005; Downs et al., 2009; Sigal and Ethier, 2009a; Sigal and Ethier, 2009b; Sigal et al., 2009a, b; Sigal et al., 2010b). Figures 1 and 2 of this article explain a subset of these concepts in greater detail.

ONH Connective Tissue Biomechanics and the Translaminar Pressure Gradient

The goal of this section is to elucidate the biomechanical determinants of the translaminar pressure gradient or the transition from intraocular pressure to retrolaminar tissue pressure experienced by the axons as they pass through the lamina cribrosa to exit the eye are illustrated and explained in Figure 2. The importance of this gradient to axonal physiology is separately discussed below. The key messages of this and the following sections are five-fold. First, energy is required for axon transport and the translaminar pressure gradient may increase the energy requirements of the RGC axons within the lamina cribrosa. Second, IOP-related stresses and strains within the ONH connective tissues are complicated and do not necessarily lead to deformation of the lamina out of the plane of the sclera. Third, scleral canal expansion that tightens the lamina within the canal and lessens posterior laminar deformation, still increases strain within the lamina. Fourth, posterior deformation of the lamina is likely not required for axon transport compromise. Fifth, IOP-related stress and strain within the ONH connective tissues may independently affect the delivery of nutrients

to the RGC axons (and therefore affect axon transport) in the presence or absence of frank laminar deformation.

The difference between Intraocular and orbital pressure establishes a set of principal "engineering" or "mechanical" *stresses* (force/cross-sectional area of the tissue bearing the load) within the ONH neural and connective tissues, the magnitude of which are determined by the level of IOP and the 3D geometry or architecture of the tissues that carry them (Burgoyne et al., 2005; Downs et al., 2009; Sigal and Ethier, 2009b). These *mechanical stresses* are separate from *physiologic stress* which we define as physical and metabolic changes within a cell in response to alterations in its environment. The direct (outward) effect of intraocular pressure on the internal limiting membrane of the ONH prelaminar tissues is resisted by the pressure within the retrolaminar optic nerve tissues (retrolaminar tissue pressure) and the outward expansion of the scleral canal which pulls the lamina cribrosa "tight" within the canal, effectively increasing its resistance to outward deformation (Bellezza et al., 2003a; Downs et al., 2009).

The important concept for this discussion is that engineering models suggest that the stresses generated by the IOP/orbital pressure difference within the scleral connective tissues are far higher than the direct (outward) stresses on the neural and connective tissues of the lamina (figure 2) (Collins, 1967;Downs et al., 2009;Sigal et al., 2005;Sigal et al., 2009b). How the ONH connective tissues respond to a given distribution of mechanical stress is determined by their material properties. A growing body of experimental (Agoumi et al., 2010;Bellezza et al., 2003a) and theoretical work (Sigal, 2009;Sigal et al., 2005;Sigal et al., 2009b) supports the concept of a laminar-scleral dynamic in which the net compliance or rigidity of the sclera exerts a large influence over the magnitude of lamina cribrosa deformation at all levels of IOP. While a previous study of ONH surface deformation in dogs suggested substantial ONH surface movement with acute IOP elevation (Morgan et al., 2002), two recent studies in which laminar deformation was measured directly, suggest little posterior laminar deformation follows acute IOP elevation in normal monkey (Bellezza et al., 2003b) (Burgoyne et al., 2009. Deformation of the Normal Monkey Optic Nerve Head (ONH) Connective Tissues Following Acute IOP Elevation Within 3D Histomorphometric Reconstructions. Invest Ophthalmol Vis Sci. 50, ARVO Abstract# 4897) and human eyes (Agoumi et al., 2010).

How the axons respond to laminar deformation, (when present) can not be separated from other axonal effects of the constituent neural and connective tissue stresses and strains. Said in another way, the components of IOP-related stress and strain that drive ONH connective tissue deformation may not be the components that influence axon transport. Glaucomatous damage to the RGC axon within the ONH may not simply occur at locations with the highest levels of IOP-related connective tissue strain or stress. Rather as neural and connective tissue stress and strain increase, axon physiology may become compromised at those locations where the translaminar tissue pressure gradient is steepest (Figure 2) and/or where the axon's energy supply is or otherwise becomes most vulnerable.

In these regards, it is important to clarify the separate concepts of the translaminar pressure difference (the difference between IOP and the retrolaminar tissue pressure) - which generates a net posterior (outward) force on the surface of the lamina and the translaminar pressure gradient (schematically shown in green in Figure 2) which is the hydrostatic pressure gradient within the neural and connective tissues of the pre-laminar and laminar regions created by the translaminar pressure difference. Note that the in-plane hoop stress transferred to the lamina from the sclera is much larger than the stresses induced by the translaminar pressure difference (Figure 2). CSF directly influences laminar position through its effect on the translaminar pressure difference. CSF may also effect scleral flange

position within the region it projects to the sclera (Figure 2), but in most eyes, because the projection of the CSF space is minimal this is not likely important (Figure 2).

IOP has a similar direct effect on laminar position, but has an additional (and potentially more important) effect through the peripapillary sclera (Figure 2). However, while the magnitude of the translaminar pressure difference may be small relative to the stresses within the connective tissues of the sclera and lamina, the axons separately experience it as the translaminar pressure gradient, the steepness of which is influenced by the thickness of the tissues over which it is experienced. In eyes with thin laminas or in which the lamina becomes thin through the course of the neuropathy,(Jonas, 2007;Jonas et al., 2003,2004;Morgan et al., 1998;Morgan et al., 2008) the translaminar pressure gradient may serve as a primary barrier to axon transport and flow within this region and an important physiologic determinant for the ONH axons and cells.

The fact that axon transport blockade within the “scleral” region of the mouse and rat ONH has been reported in multiples studies following acute and chronic IOP elevation (Danias et al., 2003; Filippopoulos et al., 2006; Howell et al., 2007; Jakobs et al., 2005; Johansson, 1983; Martin et al., 2006; Schlamp et al., 2006) is important to this discussion for the following reasons. First, it suggests that deformation of connective tissue lamina cribrosa beams is not necessary for axonal transport to be challenged in this location. Second, it suggests that the translaminar pressure gradient, alone, or in combination with the astrocyte and vascular response to it may provide an important challenge to axon transport when IOP is elevated.

Retinal Ganglion Cell Axon Transport Within the ONH

The purpose of this section is to identify the principal components of RGC axon transport within the ONH with emphasis on the delivery of nutrients required to meet its energy demands. Reviews of this subject (Anderson and Hendrickson, 1974; Barkus et al., 2008; Duncan and Goldstein, 2006; Gross et al., 2007; Minckler, 1994; Minckler et al., 1977; Morgan, 2000, 2004; Quigley and Anderson, 1976) should be consulted for greater detail.

Axon transport is energy dependant

In addition to the conduction of axon potentials, the retinal ganglion cell axons transport molecules, vesicles and organelles in both the anterograde (orthograde – away from the cell body, toward the brain) and retrograde (toward the cell body, away from the brain) directions. The axon cytoskeleton forms the molecular tracks for both anterograde and retrograde movement of motor proteins and their associated cargo in addition to maintaining RGC and axon shape. Neurofilaments, microtubules, microtubule-associated proteins (MAPs) and actin are the major constituents of the axon cytoskeleton. Microtubules are highly polarized structures made up of alpha and beta tubulin that possess a stable “minus” end close to the cell body and a “positive” end that is thought to be more unstable (Barkus et al., 2008; Duncan and Goldstein, 2006; Gross et al., 2007; Vale and Milligan, 2000). Physical disruption of the axon cytoskeleton has been reported to occur within 3 hours of acute IOP elevations and precede axon transport abnormalities in the porcine eye (Balaratnasingam et al., 2008).

All of the molecular motors for axon transport hydrolyze Adenosine-5′-triphosphate (**ATP**) to generate movement along the microtubules. Anterograde transport occurs in fast (50–400 mm/day) and slow (slow transport components A (SCa.3–3mm/day) and B (SCb 2–8 mm.day) forms. The molecular motor for anterograde transport is provided by a family of kinesin molecules. The kinesins are synthesized in the cell body and are activated on binding a cargo molecule whereupon they bind to the microtubules and commence movement.

Retrograde transport is fast (200–400 mm/day) and uses dynein as the molecular motor. Dynein is synthesized in the cell body, transported to the axon terminal by both fast and slow mechanisms. It is activated upon binding to its target molecule whereupon it commences retrograde transport along the microtubules. Recently, a second family of vesicular molecular tracks (actin filaments and myosin motors) have been proposed for both anterograde and retrograde transport (Barkus et al., 2008; Duncan and Goldstein, 2006; Gross et al., 2007).

Mitochondria move within axons using both the actin and microtubule tracks (Brown, 2003; Hollenbeck and Saxton, 2005) to achieve positions in the cell where metabolic support is required such as myelin boundaries (Bristow et al., 2002) and regions of demylenation (Mutsaers and Carroll, 1998). Mitochondrial accumulation within, and on both sides of the lamina has been reported to be a feature of normal monkey (Minckler et al., 1976), cat (Bristow et al., 2002), and human (Barron et al., 2004; Hollander et al., 1995) eyes and ascribed to the extraordinary energy requirements of this region (Andrews et al., 1999; Howell, 1997; Morgan, 2004).

Age-related alterations in the retinal ganglion cell axon

Vrabec reported the presence of swollen axons within the nerve head at the level of the lamina in cadaver eye specimen from individuals ranging in age from 46 to 80 years, with the number of swollen axons increasing with age (Vrabec, 1977). Dolman, et al (Dolman et al., 1980) confirmed this finding in patients above the age of 65, in a separate study of 300 optic nerves from humans spanning the ages of birth to 96 years. In nerves from patients in their seventh decade, three fifteenths demonstrated this finding. In nerves from patients in their eighth decade it was five fifteenths, and from those in their ninth and tenth decades, eight fifteenths. Dolman ascribed the age-related swelling of axons in their study to the combination of age related alterations in astrocytes, blood vessels and axons they described (Dolman et al., 1980).Johnson found similar swellings in the optic nerves of aged mice (Johnson et al., 1978).

The nutrient supply of the RGC axons within the ONH has not been determined

Little is known regarding the actual delivery of nutrients to the retinal ganglion cell axon within the ONH. In the retina (Wang et al., 2002; Yu et al., 2010), prelaminar (Haefliger and Anderson, 1996), laminar and retrolaminar ONH (Haefliger and Anderson, 1996) astrocyte processes invest the retinal ganglion cell axons and are presumed to be responsible for maintaining the extracellular environment - possessing uptake mechanisms for the removal of potassium and glutamate from the extracellular space as well as providing neurotrophic support. How the retinal ganglion cell axons receive the nutrients necessary to power their mitochondria has not been rigorously studied. In the retina (Wang et al., 2002; Yu et al., 2010), prelaminar ONH (Haefliger and Anderson, 1996) and brain (Gordon et al., 2007; Mulligan and MacVicar, 2004) astrocytes have direct contact with the endothelial cells of capillaries and it is presumed there is direct transfer in addition to diffusion of nutrients (Haefliger and Anderson, 1996).

Within the lamina cribrosa of the monkey and human, where each capillary is embedded within a connective tissue sheath (Figure 1), the astrocyte that envelops the beam (and is also responsible for the maintenance of its extracellular matrix), is separated from the laminar beam capillary by its basement membrane, the laminar beam extracellular matrix, the pericytes, endothelial cells and the endothelial cell basement membranes. To date, there have been no definitive studies to determine if the laminar beam astrocyte sends a process to the laminar beam capillary. If, in fact, the laminar beam astrocyte does not have a direct connection to the laminar beam capillary, this is the only place in the central nervous system

where that is the case. Several important implications follow. First IOP-related stress and/or strain may act as a hydrostatic and or compressive pressure and influence the volume flow of blood within the capillaries and/or the diffusion properties of these tissues. Second, age and/or disease related alterations in the beam and basement membrane connective tissues may enhance these effects.

IOP-related connective tissue stress and strain should affect laminar capillary blood flow

Retrobulbar blood flow within the posterior ciliary arteries is principally influenced by systemic blood pressure and vasospasm. However, IOP-related strain within the peripapillary sclera may significantly affect volume flow through the scleral branches of the short posterior ciliary arteries (Langham, 1980) (Figures 3 and 4). Thus, volume flow through the circle of Zinn Haller and the small penetrating vessels that pass anteriorly to the prelaminar nerve, transversely into the laminar insertion sites, and posteriorly to the pial branches supplying the retrolaminar optic nerve can be theoretically diminished by elevated levels of IOP-related strain (Langham, 1980). Separate from these retrolaminar effects, frank occlusion of the capillaries running within each laminar beam may occur due to tensile, compressive, or shear strains within the beam that are still within the beam's elastic limits (Figures 4 and 5). This may be more likely to occur when capillary perfusion pressure has been adversely affected by retrolaminar effects of IOP-related strain or non-IOP related factors such as nocturnal hypotension, diabetes, and retrolaminar vasospasm (Hayreh et al., 1994; Langham, 1980; Spaeth, 1977).

The effects of IOP-related connective tissue stress and strain on nutrient transport from the laminar beam capillary to the astrocyte have not been determined

In the absence of a laminar beam astrocyte footplate on the laminar beam capillary, axonal nutrition within the lamina likely requires diffusion of nutrients from the laminar capillaries, across the endothelial and pericyte basement membranes, through the extracellular matrix of the laminar beam, across the basement membranes of the astrocytes, into the astrocytes, and across their processes to the adjacent axons (Panel F, Figure 1). Chronic age-related changes in the endothelial cell and astrocyte basement membranes, as well as IOP-induced changes in the laminar extracellular matrix and astrocyte basement membranes, may diminish nutrient diffusion to the axons even in the presence of a stable level of laminar capillary volume flow.

Age related alterations in the laminar astrocyte/laminar capillary dynamic may contribute to age-related and glaucomatous axon loss

The aged ONH is more likely to have stiff connective tissues (Albon et al., 2007; Albon et al., 1995; Albon et al., 2000a; Albon et al., 2000b; Bailey et al., 1998; Brown et al., 1994; Friedenwald, 1937; Hernandez et al., 1989; Jeffery et al., 1995; Kotecha et al., 2006; Morrison et al., 1989a; Pena et al., 1996; Quigley, 1982) and a compromised blood supply (Grunwald et al., 1993; Harris et al., 2000). However, age-related increases in laminar beam thickness(Albon et al., 2000a; Hernandez et al., 1989; Hernandez et al., 1991; Kotecha et al., 2006; Morrison et al., 1989a), laminar astrocyte basement membrane thickness(Hernandez et al., 1989; Hernandez et al., 1991) and laminar extracellular matrix (ECM) stiffening(Albon et al., 2000a; Hernandez et al., 1989; Hernandez et al., 1991; Morrison et al., 1989a) should not only increase laminar beam stiffness, but also diminish nutrient diffusion from the laminar capillaries into the adjacent axons. While the concepts of age-related optic nerve axon loss(Balazsi et al., 1984; Cepurna et al., 2005; Cull et al., 2003; Morrison et al., 1990; Repka and Quigley, 1989; Sandell and Peters, 2001, 2002) and an optic neuropathy of aging (Frisen, 1991; Jonas and Grundler, 1996; Klein et al., 2006; McKendrick et al., 2007; Sandell and Peters, 2002; See et al., 2009) remain controversial, we believe that the range of physiologic stress and strain within the ONH connective tissues

experienced over a life-time are likely to be of central importance to both. Interestingly, Hogan and Zimmerman (Hogan and Zimmerman, 1969) proposed that thickening of the retrolaminar septa caused a reduction in the diffusion of nutrients from the contained capillaries to the axons, contributing to age-related loss.

Both normal and elevated levels of IOP-related strain can be expected to induce synthesis of extracellular matrix by the laminar astrocytes (Agapova et al., 2003; Agapova et al., 2001; Pena et al., 2001), which alters the material properties (and likely the diffusion properties) of the adjacent laminar trabeculae. Additionally, the laminar astrocytes and laminar capillary endothelial cells may thicken their basement membranes as a secondary response to the physical distortion induced by laminar deformation. Thickening of the basement membranes of the laminar capillary endothelial cells in response to elevated levels of IOP-related stress was reported in a poster by Hernandez's group at the 1997 ARVO meeting (Pena et al., 1997). Basement membranes of the laminar trabeculae have also been noted to thicken with age (Hernandez et al., 1989).

Disease-related alterations in the laminar astrocyte/laminar capillary/retinal ganglion cell axon dynamic may increase susceptibility to axon loss

A review of the literature supports the notion that the relationships between the laminar astrocytes, beams, beam capillaries and retinal ganglion cell axons are profoundly altered throughout the neuropathy (Downs et al., 2009; Morgan, 2000, 2004; Quigley, 1999). Until 10 years ago, descriptions of very early changes in the neuropathy of glaucoma were only sporadically reported in the monkey and human literature (Agapova et al., 2003; Harwerth et al., 1999; Pena et al., 2001; Quigley et al., 1983; Tezel et al., 1997). In recent years, however, studies of early experimental glaucoma in the mouse, rat and monkey ONH have confirmed that the 3D histomorphometric (Burgoyne et al., 2004; Downs et al., 2007; Yang et al., 2007a; Yang et al., 2009; Yang et al., 2007b) and molecular changes (Du et al., 2007; Fu and Sretavan, 2010; Fu et al., 2010; Howell et al., 2007; Mabuchi et al., 2004) within weeks of the onset of modest IOP elevations are already profound.

Figures 6, 7 and 8 summarize the ONH alterations we have histologically (Bellezza et al., 2003b; Downs et al., 2001) and histomorphometrically (Burgoyne et al., 2004; Downs et al., 2007; Yang et al., 2007a; Yang et al., 2009; Yang et al., 2007b) described in an initial group of 3 monkeys with early unilateral experimental glaucoma and recently extended to an additional group of 6 animals (Yang et al., 2010). The onset of "cupping" in monkey experimental glaucoma in the setting of modest to moderate chronic IOP elevations is defined by co-localized posterior laminar deformation and neural canal expansion that is maximum within the superior temporal and/or inferior-nasal quadrants. These phenomena are accompanied by laminar thickening, axial elongation, and outward peripapillary scleral bowing in the majority of eyes.

We have also reported preliminary evidence of laminar beam thickening (Grimm et al., 2007. 3D Quantification of Lamina Cribrosa Microarchitecture in Normal and Early Glaucomatous Monkey Eyes. Invest. Ophthalmol. Vis. Sci. 48, ARVO Abstract# 3295) at this early stage of damage. In addition, Roberts et al have reported preliminary evidence of retrolaminar septal recruitment (Roberts et al., 2009). However, separate from these phenomena, the lamina appears to actively migrate out of the sclera and into the pia within regions of the most affected eyes (Figure 8). In some instances complete "pialization" of the laminar insertion is achieved (Figure 8). Whether the driving event of this phenomenon is mechanical failure of the anterior laminar beams (pushing the lamina to seek additional connective tissue support by recruiting the retrolaminar septal tissues), loss of the anterior (scleral) blood supply or active recruitment of the retrolaminar tissues as part of "remodeling", the implications of these alterations for the blood supply of the laminar

beams, the steepness of the translaminar pressure gradient and astrocyte and axonal physiology are profound.

Laminar insertion into the pia has been observed in normal human cadaver eyes (Sigal et al., 2010a) (Girkin et al., 2010. Three-Dimensional Reconstruction of the Human Optic Nerve Head. Invest. Ophthalmol. Vis. Sci. 51, ARVO Abstract# 3854). However, laminar migration and pialization as an active component of the optic neuropathy of glaucoma (i.e. as an alteration from a previously normal state) has not been previously recognized. Our description of laminar migration and pialization within this group of 9 early experimental glaucoma monkeys as well as its contribution to the evolution of “glaucomatous cupping” in this species (Burgoyne et al., 2005; Downs et al., 2009) is in preparation and will be the subject of a future report.

Chauhan et al (Chauhan et al., 2002) described profound alterations of the ONH and scleral canal tissues as well as ERG changes in rats with 1 to 3 months exposure to chronic IOP elevation that were different from those following chronic exposure to endothelin (Chauhan et al., 2004) and appeared to relate to the magnitude of peak IOP elevation and cumulative IOP exposure of the treated eye. Johnson et al (Johnson et al., 2007) reported genomic alterations compatible with cell proliferation, immune response, lysozome, cytoskeleton, extracellular matrix and ribosome in a genomic analysis of the ONH tissues of rats after 5 weeks of chronic unilateral IOP elevation. A 2.7 fold increase in the number of cells in the ONHs of glaucoma eyes was separately confirmed by cell counts.

Howell, et al (Howell et al., 2007) reported axonal insults within the laminar regions of the DBA/2J mouse at the earliest detectable stages of optic neuropathy. This study confirmed previous work that suggested the “glial” lamina of the mouse eye was sufficient to be the site of axonal disruption (Danias et al., 2003; Filippopoulos et al., 2006; Howell et al., 2007; Jakobs et al., 2005; Schlamp et al., 2006) but did not elucidate the mechanism. Cytoskeletal, size and Glial Fibrillary Acidic Protein (GFAP) expression changes in astrocytes within regions of the porcine ONH that demonstrated axon transport abnormalities have been reported as early as 3 hours after acute IOP elevation (Balaratnasingam et al., 2008).

Taken together these data from monkey, rat and mouse eyes are compatible with the notion of a direct axonal insult by a variety of mechanisms coupled with astrocyte withdrawal of trophic support due to “preoccupation” with or “distraction” by a profoundly altered biomechanical environment (Hernandez, 2000; Hernandez and Pena, 1997; Jamara et al., 2000).

The mechanisms of retinal ganglion cell insult in glaucoma are likely multifactorial and different by region of the ONH and stage of disease

As of this writing, none of the classically proposed mechanisms of RGC axonal insult within the ONH can be discounted. These include: 1) ischemia through a variety of mechanisms (the traditional vascular hypothesis (Fechtner and Weinreb, 1994)), 2) physical compression of the axons secopndary to the deformation of intact laminar trabeculae (the traditional mechanical hypothesis (Fechtner and Weinreb, 1994)), and 3) spontaneous axonal compression secondary to tissue pressure differences across the intact lamina cribrosa (Yablonski and Asamoto, 1993). All remain plausible either acting individually or in combination within different regions of the nerve head at a given time and/or at different stages of the disease.

Recent work by a series of authors (Band et al., 2009; Berdahl et al., 2008a; Berdahl et al., 2008b; Morgan et al., 2008; Morgan et al., 1995; Ren et al., 2009a; Yablonski and Asamoto,

1993) strongly suggest that acquiring the energy necessary to maintain the physical integrity of the axon and its active transport mechanisms through the translaminar pressure gradient may represent the “final common pathway” of damage to the axon in the face of a variety of insults. Steepening this gradient by increasing its magnitude or “thinning” the region through which the axons experience it, may increase axonal susceptibility (Jonas, 2007; Jonas et al., 2004; Ren et al., 2009b).

However, there is also a growing realization that the optic neuropathy of glaucoma is an active process in which the scleral fibroblasts, laminar astrocytes and glia remodel their environment to better suit the altered biomechanical milieu in which they find themselves (Agapova et al., 2003; Hernandez, 2000; Hernandez et al., 2008; Hernandez and Pena, 1997; Johnson et al., 2007; Johnson and Morrison, 2009; Kirwan et al., 2004; Roberts et al., 2009; Varela and Hernandez, 1997; Vernon et al., 2008). In some ONHs, this may represent a small shift out of the biological continuum of aging (Burgoyne and Downs, 2008) that leads to an increase in the rate and magnitude of age-related axon loss such that they become clinically recognizable as glaucomatous (See et al., 2009). In others, this is a profound shift that may leave the astrocytes in a “better” biomechanical environment but distracted from their trophic task of retinal ganglion cell axon maintenance.

Future Directions

The concepts of the retinal ganglion cell axon being abandoned due to ONH astrocyte “distraction in addition to its being damaged by astrocyte “activation” should continue to be explored (Hernandez and Pena, 1997). Within this context, as well as within the larger discussion of retinal ganglion cell injury mechanisms - above, the following research questions are important.

1. **Why do primates have a connective tissue lamina and does this influence axonal susceptibility?** Is a connective tissue lamina “protective” of the axons; is it protective in bigger eyes or just longer living eyes, or both? If it is protective, what are the mechanisms and why are they important? Are rates of age-related axon loss in mice and rats higher than in tree shrew, pig and monkey? Are there detectable differences in the susceptibility of young and old mice and rats to acute and chronic IOP elevations and is this susceptibility more than in young and old tree shrews, pigs and monkeys?

2. **Do astrocytes have processes on the laminar capillaries in species with connective tissue laminar beams? Hernandez suggested that there were laminar processes and that they were withdrawn in human glaucoma in a single article that did not employ electron microscopic characterization (**Hama et al., 2004; Varela and Hernandez, 1997; Wilhelmsson et al., 2006). What is the relationship of the laminar astrocytes to the laminar capillaries in mice and rats compared to tree shrews pigs and monkeys? Does this change with age and early disease? Do astrocytes immediately withdraw their processes from the laminar beam capillaries and axon bundles as part of their initial response to insult? Can we intervene to stabilize these processes in a beneficial way?

3. **What are the important components of axonal nutrition within the ONH?** How are these altered by age and disease? Do the laminar beam connective tissues (including the beam and basement membrane ECM) influence nutrient diffusion and if so how? Can IOP-related stress and strain within the beams, (separate from their ECM) influence nutrient diffusion? If you make the laminar beam stiffer, is there a clinically important effect on diffusion? Are these relationships changed in age and disease?

4. **Can IOP-related Stress and Strain within the ONH connective tissues directly diminish the volume flow of blood within the contained scleral branches of the posterior ciliary arteries and the laminar beam capillaries?** What are the mechanisms of laminar beam capillary autoregulation? (Liang et al., 2010) Are they altered in aging and do they falter early in the optic neuropathy of glaucoma?

5. **Do astrocytes migrate away from the beams early in the optic neuropathy of glaucoma?** While Hernandez is often cited on this point, in fact the evidence for this is based on a single figure within a single article (Varela and Hernandez, 1997), is not quantitative, nor was electron microscopy employed (Hama et al., 2004; Wilhelmsson et al., 2006). A second assessment of astrocyte migration in cell culture was reported by Tezel (Tezel et al., 2001). This issue is important and needs to be rigorously revisited. If this is true, are the cells that migrate new cells (the result of astrocyte proliferation (see below) - leaving laminar beam astrocytes and their processes in place) or are these the cells that were previously anchored to the beam and have truly migrated away from it? If so what happens to their beam and axon processes? (Hernandez suggested they were withdrawn but the figure that supports this finding is based on immunohistochemistry and can not be interpreted (Varela and Hernandez, 1997))

6. **Do astrocytes proliferate early in the neuropathy of glaucoma?** A growing body of evidence supports the notion that astrocytes within the laminar region of mice, rats and monkeys with experimental glaucoma as well as human glaucoma eyes proliferate (Johnson et al., 2007; Prasanna et al., 2002). If this is true, is withdrawal of laminar beam and axon bundle processes part of this process and is axonal nutrition compromised as a result.

7. **How many different cell types are there in the ONH and do they have functional roles that separately contribute to the optic neuropathy of glaucoma?** In addition to oligodendrycytes and microglia, two types of ONH astrocytes have been identified (Ye and Hernandez, 1995) as well as a separate cell type known as the lamina cribrosacyte (Hernandez et al., 1988). Are there species differences in these subtypes? Are there regional distribution differences? Do these cells consistently demonstrate different patterns of cellular change early in the optic neuropathy of glaucoma?

8. **Can we develop in vivo and/or clinical measures of the axon cytoskeleton and transport to assess acute and chronic perturbations of the RGC axon prior to frank structural loss?** A series of investigators are working on in vivo assessments of the axon cytoskeleton (Fortune et al., 2008a; Fortune et al., 2008b; Huang and Knighton, 2005; Huang et al., 2006)(Fortune et al., 2009. The Effect of Acute Intraocular Pressure Elevation on Peripapillary Retinal Thickness, Retinal Nerve Fiber Layer Thickness and Retardance. Invest Ophthalmol Vis Sci. 50, ARVO Abstract# 5824) and transport (Kanamori et al., 2009) (Wang et al., 2010. Fluorescent Markers of Retinal Astrocytes, Ganglion Cells, Axons and Axonal Transport Visualized in vivo by Confocal Scanning Laser Ophthalmoscopy. Invest. Ophthalmol. Vis. Sci. 51, ARVO Abstract# 2144) that will provide important experimental and clinical tools to address the above questions.

9. **Can a “glaucomatous” optic neuropathy be induced by a primary non-IOP-related insult such as ischemia, immune-mediation or experimental CSF-lowering alone?** While endothelin has been proposed to be a model of ischemic optic neuropathy in a variety of species (Chauhan et al., 2004; Cioffi et al., 1995; Orgul et al., 1996a; Orgul et al., 1996b), a robust literature has called into question its actual mechanisms (He et al., 2007; Murphy et al., 2010; Rao et al., 2008;

Stokely et al., 2002; Wang et al., 2008; Yorio et al., 2002)and there are no definitive studies that establish the “glaucomatous” nature of the neuropathy (Chauhan et al., 2004; Oku et al., 1999). One paper has reported the “glaucomatous” loss of retinal ganglion cells in rats following systemic exposure to retinal heat shock proteins, but documentation of the “glaucomatous” nature of the neuropathy has not been provided (Wax et al., 2008). Because axon loss alone and pre-laminar forms of cupping (Burgoyne and Downs, 2008; Burgoyne et al., 2005; Yang et al., 2007a), alone, are not pathognomonic for a “glaucomatous” optic neuropathy (see above), the following questions should apply to each of these models. What constitutes a “glaucomatous” optic neuropathy? To what experimental paradigm should these models be compared? What elements of the neuropathy need to be present to call it “glaucomatous”?

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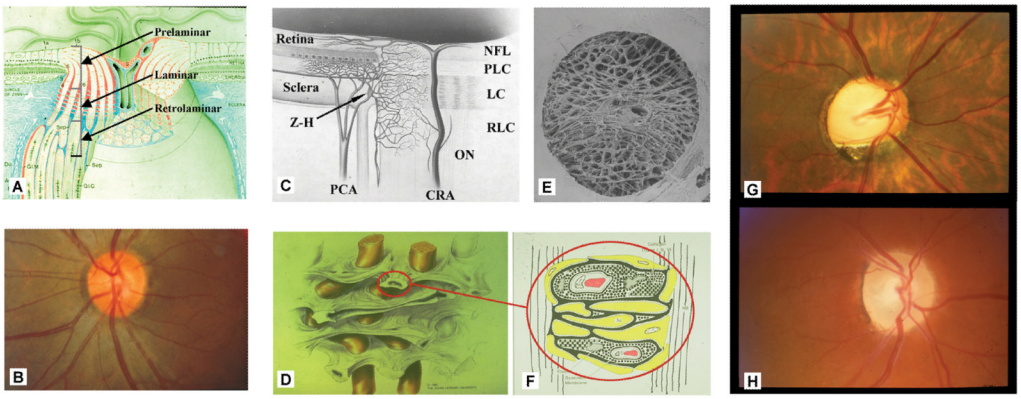

Figure 1. Glaucoma, cupping and axonal insult within the optic nerve head (ONH)

The ONH is made up of prelaminar, laminar and retrolaminar regions (A). Within the clinically visible surface of the Normal ONH (referred to as the optic disc) (B), central retinal vessels enter the eye and RGC axons appear pink due to their capillaries (which are principally supplied by branches from the posterior ciliary arteries (PCA) in (C). The primary site of RGC axon insult in glaucoma is within the lamina cribrosa (schematically depicted with axon bundles in (D), isolated by trypsin digest in a scanning electron micrograph in (E) and drawn with stippled extracellular matrix (ECM), central capillary (red) and surrounding astrocytes (yellow with basement membranes in black) (F). Blood flow within the ONH, while controlled by autoregulation, can be affected by non IOP-related effects such as systemic blood pressure fluctuation and vasospasm within the retrobulbar portion of the PCAs. Additional IOP-induced effects may include compression of PCA branches within the peripapillary sclera (due to scleral stress and strain) and compression of laminar beam capillaries reducing laminar capillary volume flow (C and F) (Langham, 1980). There is no direct blood supply to the axons within the laminar region. Axonal nutrition within the lamina (F) requires diffusion of nutrients from the laminar capillaries, across the endothelial and pericyte basement membranes, through the ECM of the laminar beam, across the basement membranes of the astrocytes, into the astrocytes, and across their processes to the adjacent axons (vertical lines). Chronic age-related changes in the endothelial cell and astrocyte basement membranes, as well as IOP-induced changes in the laminar ECM and astrocyte basement membranes may diminish nutrient diffusion to the axons in the presence of a stable level of laminar capillary volume flow. The clinical manifestation of IOP-induced ONH structural change is most commonly “deep cupping” (G) but in some eyes cupping can be shallower accompanied by pallor (H). Z-H = circle of Zinn-Haller; PCA= posterior ciliary arteries; NFL = nerve fiber layer; PLC = prelaminar region; LC = lamina cribrosa; RLC = retrolaminar region; ON = optic nerve; CRA = central retinal artery. *(A) Reprinted with permission from Arch Ophthalmol (*Anderson, 1969*); (C) reprinted with permission from The Glaucomas. St. Louis: Mosby; 1996:177–97 (*Cioffi and Van Buskirk, 1996*); (D) reprinted with permission from Optic Nerve in Glaucoma. Amsterdam: Kugler Publications; 1995:15–36 (*Quigley, 1995b*); (E) reprinted with permission from Arch Ophthalmol (*Quigley et al., 1990*); (F) reprinted with permission from Arch Ophthalmol (*Morrison et al., 1989b*)*

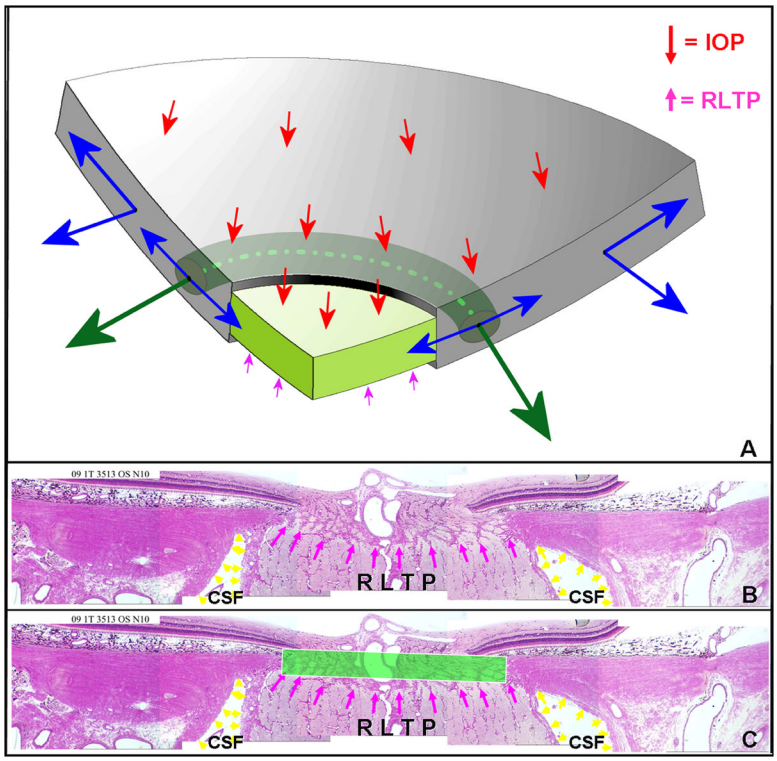

Figure 2. Principle distribution of forces, pressures and the translaminar pressure gradient within the optic nerve head (ONH)

A. Cut-away diagram of intraocular pressure (IOP)-induced mechanical stress in an idealized spherical scleral shell with a circular scleral canal spanned by a more compliant lamina cribrosa. In this case, the majority of the stress generated by IOP/orbital pressure difference (red arrows on the inner surface of the sclera) is transferred into a hoop stress borne within the thickness of the sclera and lamina (blue arrows) that is concentrated circumferentially around the scleral canal (green arrows). **B.** Note that the pressure behind the lamina is not simply CSF pressure but is retrolaminar tissue pressure **(RLTP)** which has been demonstrated to be approximately 0.82 × CSF + 2.9 mm Hg by Morgan, et al in dogs (Morgan et al., 1998) **C.** The difference between IOP and the retrolaminar tissue pressure is the translaminar pressure difference which generates both a net posterior (outward) force on the surface of the lamina (the red arrows over the lamina) and a hydrostatic pressure gradient (the translaminar pressure gradient - schematically shown in green) within the neural and connective tissues of the pre-laminar and laminar regions. **Note that the in-plane hoop stress transferred to the lamina from the sclera is much larger than the stresses induced by the translaminar pressure difference.** CSF directly influences laminar position through its effect on the translaminar pressure difference. CSF may also effect scleral flange position within the region it projects to the sclera (Figure 2), but in most eyes, because the projection of the CSF space is minimal this is not likely important (the CSF space within Panels B and C in this figure is greatly expanded due to perfusion fixation). IOP has a similar direct effect on laminar position, but has an additional (and potentially more important) effect on laminar position through the peripapillary sclera. However, while the magnitude of the translaminar pressure difference may be small relative to the stresses within the sclera and lamina, the axons experience it as the translaminar pressure gradient the steepness of which is influenced by the thickness of the tissues over which it is experienced. The translaminar pressure gradient, as such, may serve as a primary barrier to

axon transport and flow within this region and an important physiologic determinant for the ONH axons and cells. (Panel A is modified from Downs et al, 2009).

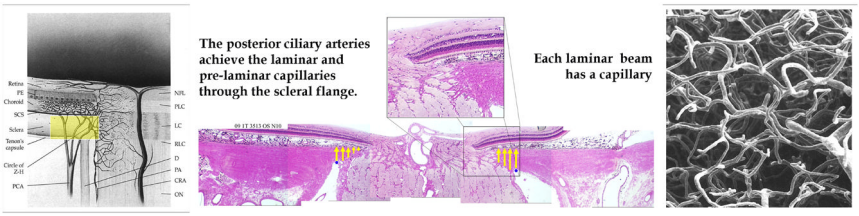

Figure 3. The volume flow of blood within the posterior ciliary arteries should be affected by IOP-related stress and strain within the peripapillary sclera and scleral flange

The posterior ciliary arteries pass through the peripapillary sclera (yellow, left and center panel) immediately adjacent to the scleral portion of the neural canal. We refer to this portion of the sclera as the scleral flange (yellow, middle panel) (Yang et al., 2005. Neural Canal and Peripapillary Scleral Alterations Within Three-Dimensional Reconstructions of Early Glaucoma Monkey Optic Nerve Heads. Invest. Ophthalmol. Vis. Sci. 46, ARVO Abstract# 3511). The sclera thins here to accommodate an expansion of the neural tissues that occurs in a highly eye-specific fashion. While the large penetrating vessels to the choroid are outside of the flange, the circle of Zinn-Haller and the penetrating branches that pass to the pre-laminar, laminar and retrolaminar nerve pass directly through these tissues and are therefore subject to the compressive effects of their contained mechanical stress and strain. Within the lamina cribrosa, there is no direct blood supply to the axons or the axon bundles. Each laminar beam contains a capillary (Panel F, Figure 1) which are here shown in a vascular casting of a monkey eye (Cioffi and Van Buskirk, 1996). *(Left and right panels reprinted with permission from The Glaucomas. St. Louis: Mosby; 1996:177–97.(*Cioffi and Van Buskirk, 1996*)*

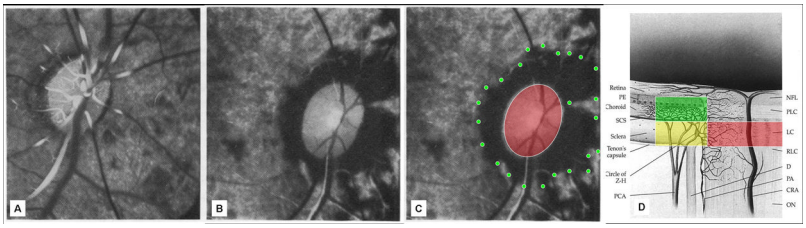

Figure 4. Hayreh (Hayreh et al., 1970) demonstrated sensitivity of the peripapillary choroidal circulation (green) to acute IOP elevation in the monkey ONH

Fluorescence fundus angiogram of the right eye of a cynomologus monkey after experimental central retinal artery occlusion at normal (A) and 70 mm Hg IOP (B and C). The non perfused region of the peripapillary choroid is highlighted in green in (C). We and others have hypothesized that IOP-related stress and strain within the scleral flange (Figure 3) may contribute to this phenomenon and that similar effects may occur within the laminar capillary beds (red) (C and D). Panels A and B are reproduced with permission from the British Journal of Ophthalmology (Hayreh et al., 1970). *(Panel D reprinted with permission from The Glaucomas. St. Louis: Mosby; 1996:177–97*. (Cioffi and Van Buskirk, 1996)

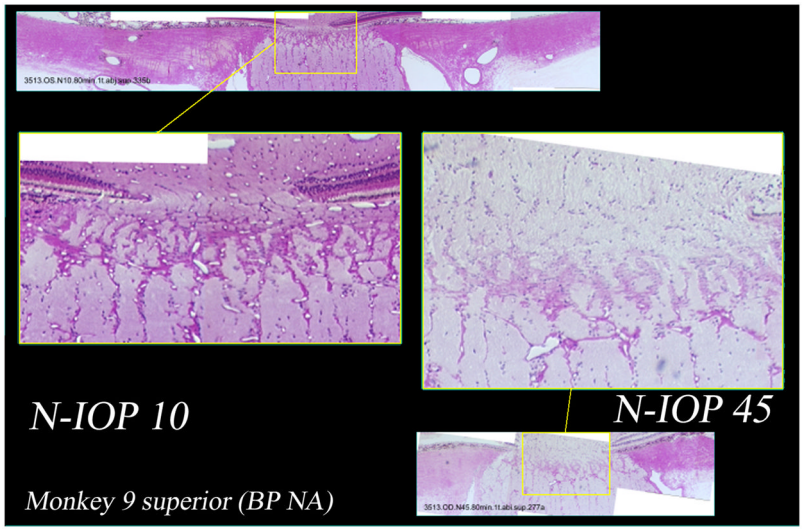

Figure 5. Histologic sections from the superior scleral canal of both eyes of a normal monkey perfusion fixed with one eye at IOP 10 mm Hg (middle left and above) and one eye at IOP 45 mm Hg (middle right and below)

Higher magnification demonstrates patent prelaminar, laminar, and retrolaminar capillaries in the IOP 10 eye (middle left). However in the IOP 45 eye (middle right), the prelaminar and anterior laminar capillaries do not appear patent, with only the posterior laminar and retrolaminar capillaries open.

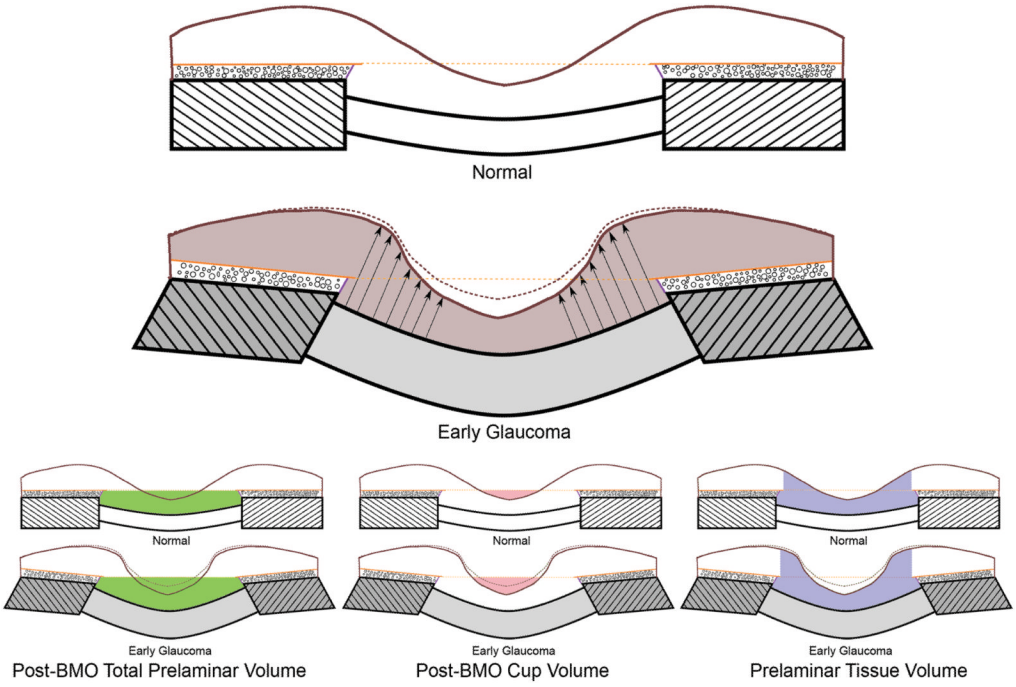

Figure 6. ONH neural and connective tissue changes at the onset of CSLT-detected ONH surface change

These findings were initially reported in n=3 monkeys (Yang et al., 2007a) and have recently been expanded to n=9 animals (Yang et al., 2010). Normal lamina cribrosa (unhatched), scleral flange (hatched), prelaminar tissue (beneath the internal limiting membrane - brown line), Bruch's membrane (solid orange line), BMO zero reference plane (dotted orange line), Border tissue of Elschnig (purple line), choroid (black circles) are schematically represented in the upper illustration. Changes in EG are depicted below. We have previously reported the bowing of the lamina and peripapillary scleral flange and thickening of the lamina in these same EG eyes (grey shading) (Yang et al., 2007b). These connective tissue changes underlie posterior deformation of the ONH and peripapillary retinal surface (dotted brown to solid brown ILM) that occurs in the setting of thickening (arrows) not thinning of the prelaminar neural tissues (brown shading) in EG. EG eye expansion of all three volumetric parameters is depicted below. The interactions between these parameters are important. While expansion of the cup and deformation of the surface are clinically detectable at this early stage of the neuropathy, because they occur in the setting of prelaminar tissue thickening, (not thinning), we believe that expansion of Post-BMO Total Prelaminar Volume (due to ONH connective tissue deformation) drives these findings. Thus, cupping in these nine EG eyes is "laminar" in origin, without a significant "prelaminar" component (Yang et al., 2007a).

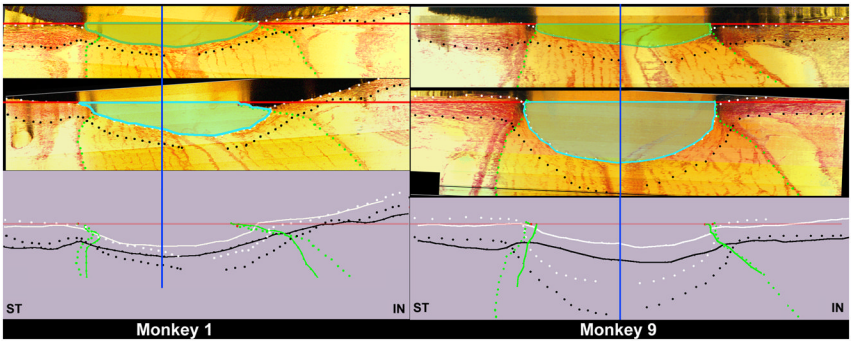

Figure 7. The range of ONH connective tissue deformation (cupping) at the onset of CSLT-detected ONH surface change in nine monkeys with unilateral experimental glaucoma (Yang et al., 2010)

Minimal (Monkey 1, left) to maximum deformation (Monkey 9, right) within the 9 animals is depicted. The following ONH landmarks are delineated within representative superior temporal (ST) to inferior nasal (IN) digital sections from the normal (upper panel) and early experimental glaucoma (EEG) (middle panel) eye of both animals: anterior scleral/laminar surface (white dots), posterior scleral/laminar surface (black dots), neural boundary (green dots), NCO reference plane (red line) and NCO centroid (vertical blue line). Delineated points for the normal (solid lines) and EEG eye of each eye are overlaid relative to the NCO centroid in the lower panel. For each animal, the area under the NCO reference plane and above the anterior laminar surface Post-NCO Total Prelaminar Volume is outlined in both the normal (darker green) and EEG (light blue) eye for qualitative comparison. The overlaid delineations for both eyes of each animal (lower panels) suggest that expansion of this area is due to posterior laminar deformation and neural canal expansion from its onset (Monkey 1) and that these two phenomena continue through more advanced stages of connective tissue deformation and remodeling (Monkey 9). As such,“glaucomatous” deformation of the ONH connective tissues is present early and progresses early in the neuropathy (Burgoyne and Downs, 2008; Yang et al., 2007a). These phenomena are accompanied by regional laminar thickening and outward bowing of the peripapillary sclera in both of these animals and most of the 9 EEG eyes. In addition, in both monkeys laminar migration to the point of partial pialization has occurred on the inferior side (right side of each schematic) of the canal (see Figure 8). (Yang et al., 2010. Optic Nerve Head Lamina Cribrosa Insertion Migration and Pialization in Early Non-Human Primate Experimental Glaucoma. Invest. Ophthalmol. Vis. Sci. 51, ARVO Abstract# 1631)

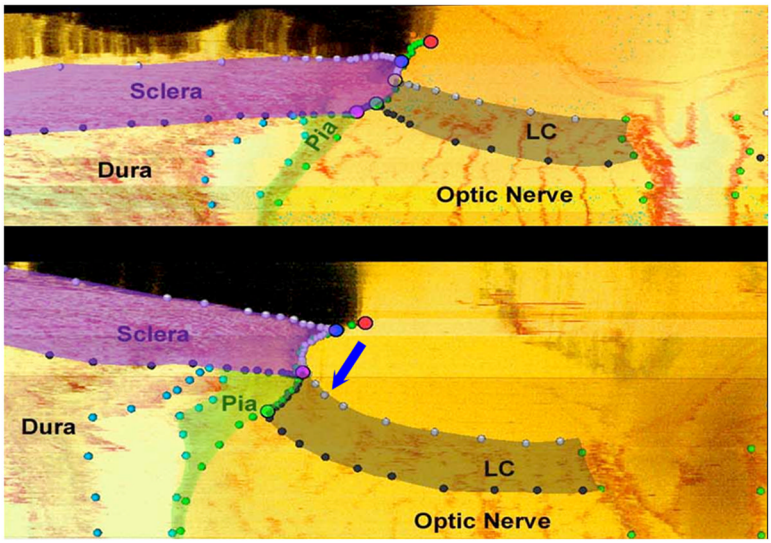

Figure 8. The pathophysiology of early experimental glaucomatous damage to the monkey ONH includes not only "thickening" but regional "migration" of the laminar insertion away from the sclera to the point that "complete pialization" of the laminar insertion is achieved in a subset of eyes

Neural canal landmarks (**Red** – Neural Canal Opening (end of Bruch's Membrane); **Blue** – Anterior Scleral Canal Opening; **Yellow** – Anterior Laminar Insertion; **Green** – Posterior Laminar Insertion; **Purple** – Posterior Scleral Canal Opening) and segmented connective tissue (*dark grey* - lamina cribrosa; *purple* - peripapillary sclera; *light green* - pial sheath) within digital section images from the inferior region of the normal (**top**) and the contralateral early experimental glaucoma (**bottom**) ONH of a representative monkey. Note that in most normal monkey eyes, the lamina inserts into the sclera as is demonstrated in this monkey's normal eye (top). However at an identical location in the early experimental glaucoma eye of this animal (bottom) in addition to the lamina being thickened and posteriorly deformed, the laminar insertion has migrated outward such that both the anterior and posterior lamina effectively insert into the pial sheath. While regions of laminar insertion into the pia have been reported in normal human eyes (Sigal et al., 2010a), these findings are the first to suggest that active remodeling of the laminar insertion from the sclera into the pia is part of the pathophysiology of "glaucomatous" ONH damage. This phenomenon when present has important implications for the mechanism of axonal insult within these regions. (These data were reported by Hongli Yang, PhD at the 2010 ARVO meeting and are currently in preparation for publication, Yang et al., 2010. Optic Nerve Head Lamina Cribrosa Insertion Migration and Pialization in Early Non-Human Primate Experimental Glaucoma. Invest. Ophthalmol. Vis. Sci. 51, ARVO Abstract# 1631).