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Research article

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

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ABSTRACT

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

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

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

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

While Dr. Epstein dedicated his research career towards elucidating our understanding of trabecular meshwork (TM) and aqueous outflow, as well as how to lower IOP by targeting the TM, he was also supportive of those of us who chose to study and potentially reverse the damage to the optic nerve. Almost three decades before the National Eye Institute announced its Audacious Goals Initiative to regenerate the axons of the optic nerve, Dr. Epstein wrote, "It is irrefutable that one would wish to prevent and reverse optic nerve damage with one's treatments, irrespective of curing the trabecular abnormality. This must be, in truth, a lofty and long-range goal that may require precedent knowledge from other areas of neurobiology and, in particular, those related to nerve regeneration." (Epstein, 1987). The varying susceptibility of the optic nerve is likely due to many different causes, and certainly much effort has been dedicated to uncovering the reasons for this phenomenon. A related concept in neurodegenerative diseases, selective vulnerability, is very much applicable to glaucoma. Selective vulnerability in the nervous system refers to the fact that subpopulations of neurons may be more or less vulnerable in

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response to injury (Saxena and Caroni, 2011). For example, in Parkinson's disease, the dopaminergic neurons of the substantia nigra selectively degenerate. In amyotropic lateral sclerosis, the upper and lower motor neurons are selectively vulnerable. For glaucoma, the retinal ganglion cells are the neuronal class most susceptible to various stressors such as elevated IOP. However, the potential mechanisms underlying selective vulnerability of neuronal subpopulations in neurodegenerative diseases are complex, multifactorial, and not yet completely understood. The same certainly holds true for glaucoma.

1.1. Are large RGCs more susceptible to injury?

While it is generally accepted that RGCs are the most vulnerable neuronal class in glaucoma, there has been controversy in the field as to whether certain RGC types are more or less vulnerable to IOP-induced injury. Earlier work in non-human primates and human tissue supported the concept that RGCs with the largest cell bodies and axons were the most susceptible to injury (Glovinsky et al., 1991; Quigley, 1999; Quigley et al., 1988, 1987). In the primate, two types of RGCs are the midget cells that comprise the P visual pathway ("P" cells) and the parasol cells that comprise the M visual pathway ("M" cells). Parasol cells have large receptive fields, high luminance and contrast sensitivity, and lack spectral sensitivity. Midget cells have smaller receptive fields, lower luminance sensitivity, and have spectral sensitivity. The first hint of selective loss among RGC types was from optic nerve axon counts in the human and non-human primate. When compared to normal optic nerves, glaucomatous optic nerves had greater loss of large diameter axons (Kerrigan-Baumrind et al., 2000; Quigley et al., 1988, 1987). Subsequent work examining RGC size and rates of cell death in whole mount retina suggested that there was a greater reduction of larger diameter RGCs, which presumably give rise to larger diameter axons (Glovinsky et al., 1991). Quigley's group went on to show that axonal transport to the magnocellular layers was more impaired than to the parvocellular layers of the dorsal lateral geniculate nucleus in non-human primates with chronic IOP elevation (Dandona et al., 1991). Indeed, in a postmortem study of the lateral geniculate nucleus from glaucoma and control patients, the mean magnocellular cell density for the glaucoma group was significantly less than that for the control group, whereas there was no difference among groups in the parvocellular layer (Chaturvedi et al., 1993). Taken together, these experiments suggested that parasol cells of the M pathway are more susceptible to IOP-induced injury than midget cells of the P pathway, although one major caveat is that there was no definitive identification of parasol or midget cells as these studies relied on size classification alone. Indeed, one major critique is that the dendritic arbor and soma size of parasol and midget cells varies greatly depending on retinal eccentricity (reviewed in (Sample, 2001)). Therefore, broadly speaking, one could argue that RGCs with larger somata and larger axons were more vulnerable, but this did not necessarily indicate selective vulnerability of a specific RGC type.

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

More recent investigations examining the function of these pathways are also conflicting with respect to the hypothesis that large field RGCs may be more vulnerable in glaucoma. Certainly, human autopsy studies and primate experimental glaucoma models suggested that neurons in the M layers of the LGN were more vulnerable than those in the P layers (Chaturvedi et al., 1993; Weber et al., 2000). Using fMRI, Zhang and colleagues found that early stage glaucoma patients were less responsive to transient achromatic stimuli than to sustained chromatic stimuli in the magnocellular layers of the LGN and the superficial layer of the superior colliculus (SC) but not in the P layers or cortical visual areas (Zhang et al., 2016). The authors conclude that early stage glaucoma causes selective functional loss of the larger cells in the human LGN and SC, specifically to stimuli modulated at high temporal frequencies. In contrast, psychophysical testing using a low-spatial-frequency contrast sensitivity approach revealed that there was no selective loss of M or P function, nor greater loss of sensitivity of larger-field RGCs (McKendrick et al., 2007). One caveat in interpreting all of these studies, both experimental animal models and human studies, is that cross comparisons are challenging because of variations in the level of IOP elevation, stage of degeneration, specificity of measurement tool, and, in the case of experimental glaucoma, the strain or species and the model used.

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

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

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