Neuroprotection:

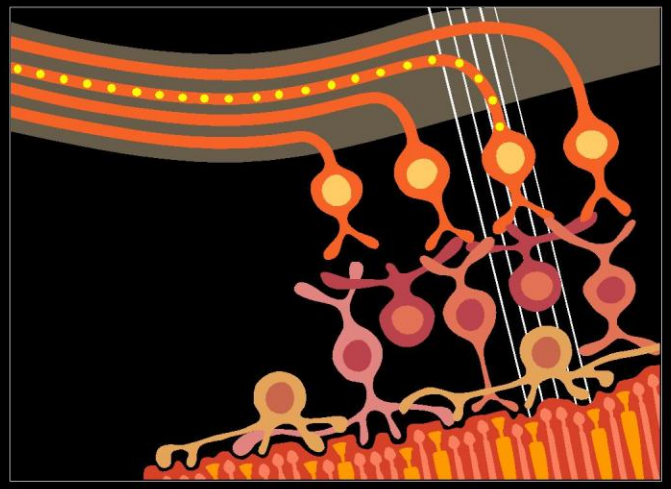
Beyond IOP

Introduction

- Glaucoma is a group of optic neuropathies
  - Retinal ganglion cell (RGC) death results in visual field loss
- Elevated intraocular pressure (IOP) is a primary risk factor and a target for therapy
  - Open-angle glaucoma (OAG) and normal-tension glaucoma (NTG)
- Pressure-independent mechanisms also involved (Flammer and Orgul, 1998)

Flammer and Orgul. *Prog Retin Eye Res.* 1998.

Introduction



- Preservation of vision is ultimate therapeutic goal
  - Target cell to protect vision is the RGC
- Risk of blindness in treated glaucoma greater than expected
  - 27% probability of unilateral blindness after 20 years (Hattenhauer et al,1998)
- There must be pathologic factors beyond IOP involved in the etiology of glaucoma

Hattenhauer et al. *Ophthalmology*. 1998.

Why Isn't IOP Lowering Enough?

- Evidence that other factors are important in the pathogenesis of glaucoma:
  - Glaucomatous damage can progress despite adequate IOP lowering
  - Glaucomatous damage may occur in individuals with IOPs in the normal range (eg, NTG)
  - Elevated IOP does not always lead to glaucomatous optic neuropathy (eg, OHT)

European Glaucoma Society Definition of Glaucoma

- Chronic, progressive optic neuropathy
- Characterized by morphological changes at the optic nerve head and retinal fiber layer
- Associated with progressive RGC death and visual field loss

European Glaucoma Society. *Terminology and Guidelines for Glaucoma,* 2nd ed. 2003.

Neuroprotective Therapies

Rationale for Neuroprotective Strategy in Glaucoma

- Neuronal cell death is the final common pathway of all glaucomatous impairment
- Disease is one of progressive neurodegeneration
  - Not dysfunctional IOP regulation
- Efforts to develop novel therapies
  - Protect RGCs independently of IOP

Neuroprotection in Glaucoma: Definition

- Neuroprotection is a therapeutic paradigm for slowing or preventing the death of RGCs and their axons to maintain their physiologic function
- Aims to block primary destructive events or enhance survival mechanisms of the RGCs or optic nerve fibers (Weinreb and Levin, 1999)

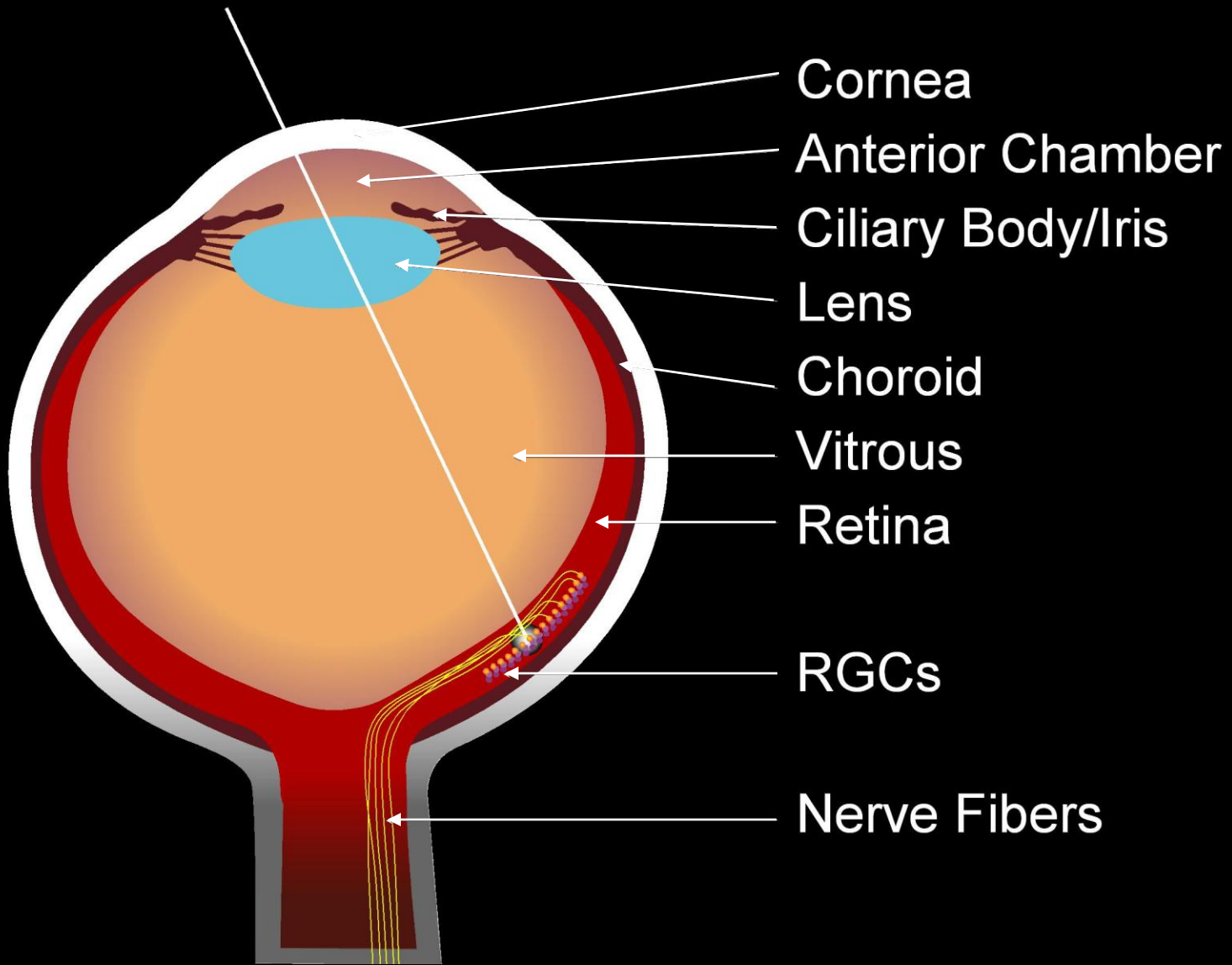
Weinreb and Levin. *Arch Ophthalmol*. 1999.

Neuroprotection in Neurological Diseases

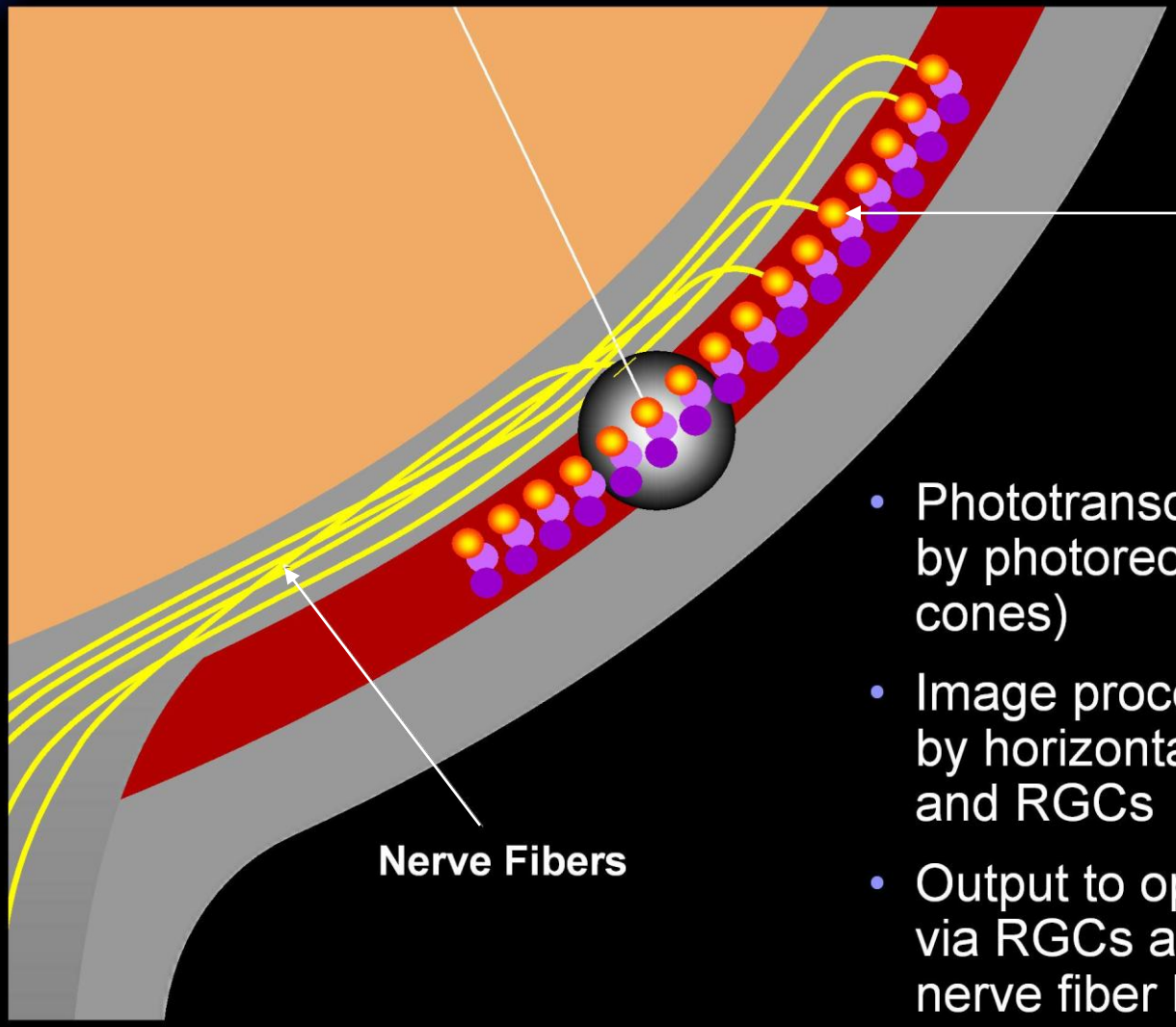
- Initially investigated for central nervous system (CNS) disorders
  - Resulted in approved therapies
- Evaluated for glaucoma
  - Heralded as having the potential to revolutionize the future treatment of the disease

Characteristics of Glaucomatous Optic Neuropathy

The Visual Pathway

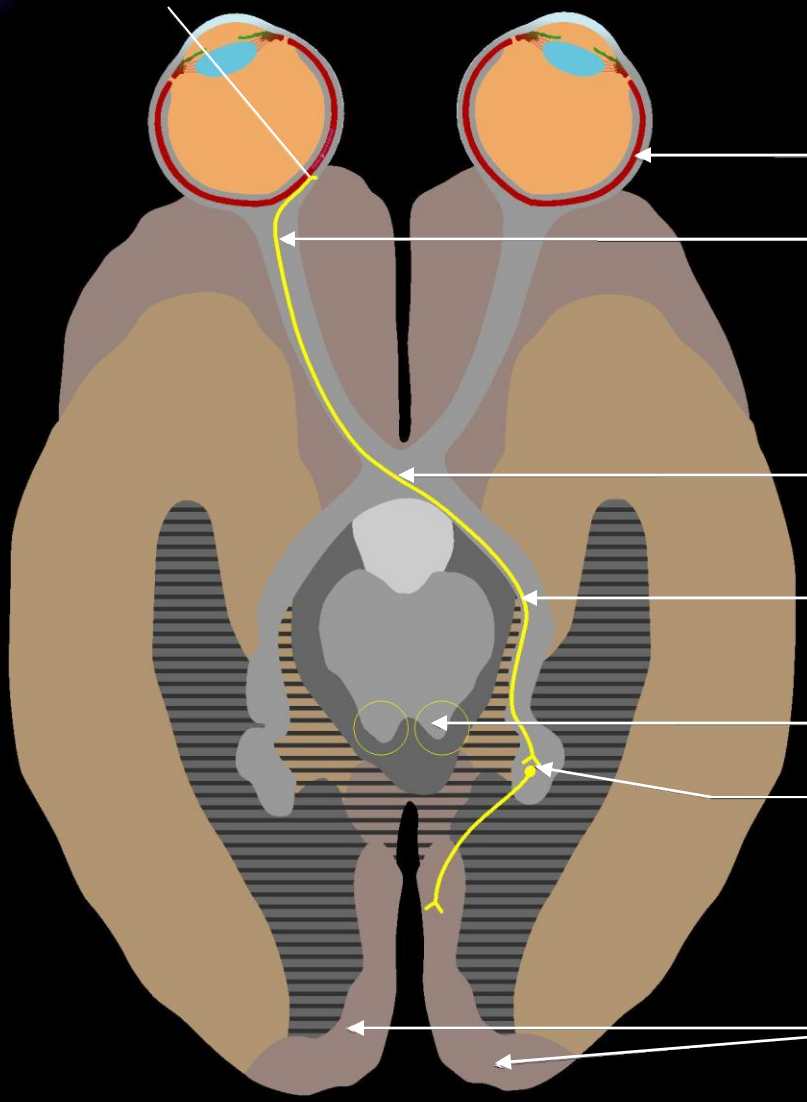


The Visual Pathway



- Phototransduction:
  by photoreceptors (rods and cones)
- Image processing:
  by horizontal, bipolar, amacrine, and RGCs
- Output to optic nerve:
  via RGCs and
  nerve fiber layer

The Visual Pathway



Retina

Optic Nerve

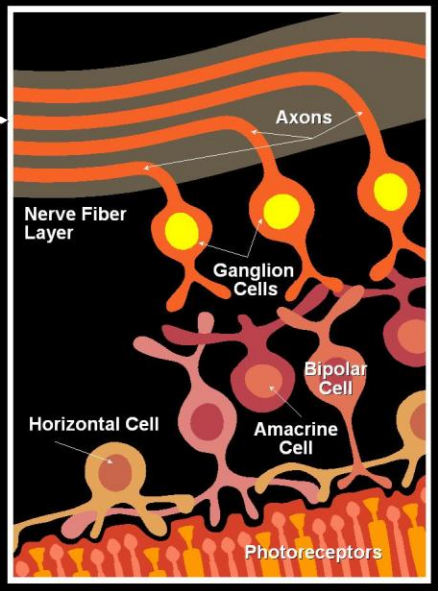
Optic Chiasm

Optic Tract

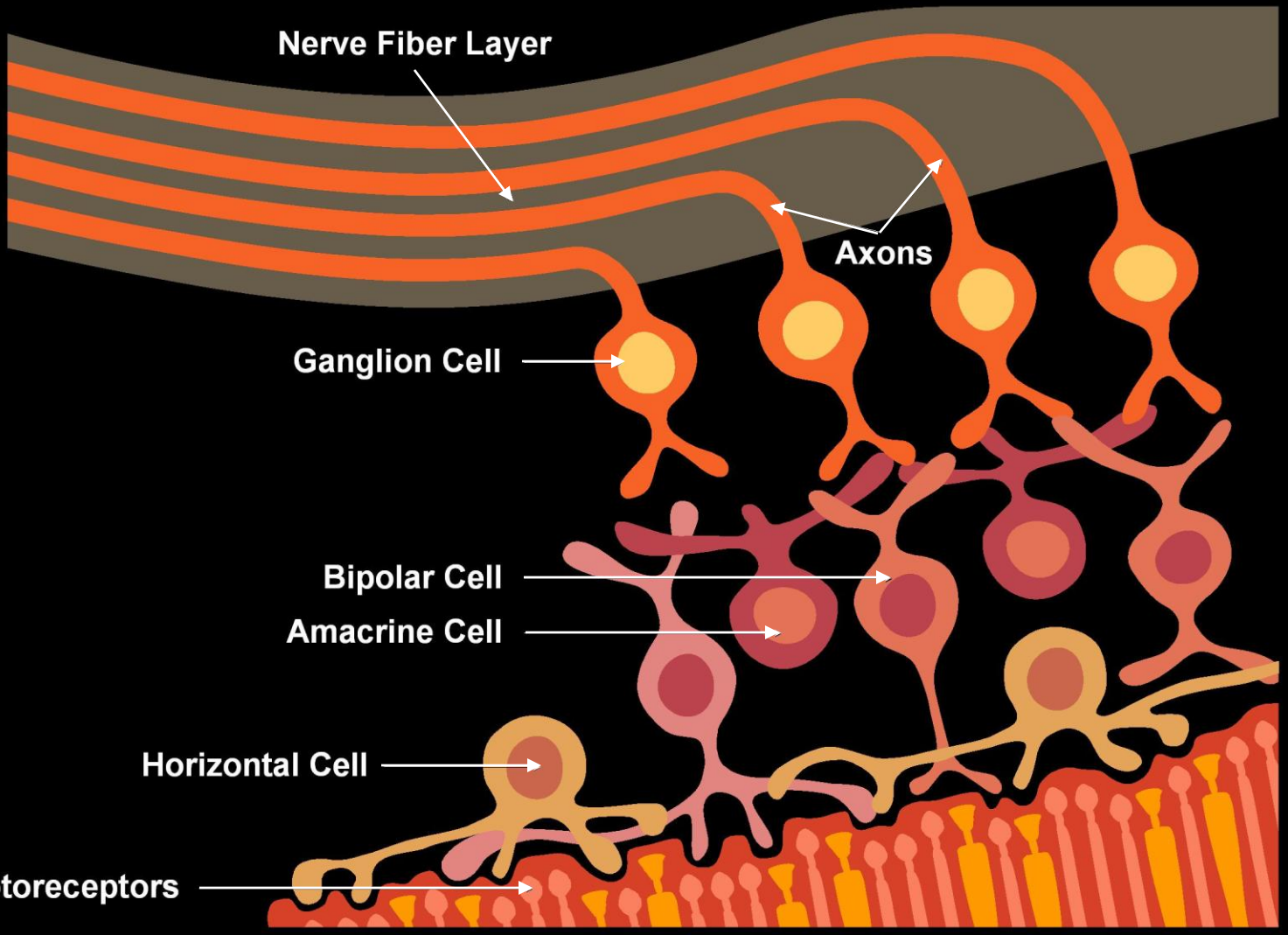
Optic Tectum

Lateral Geniculate Nucleus

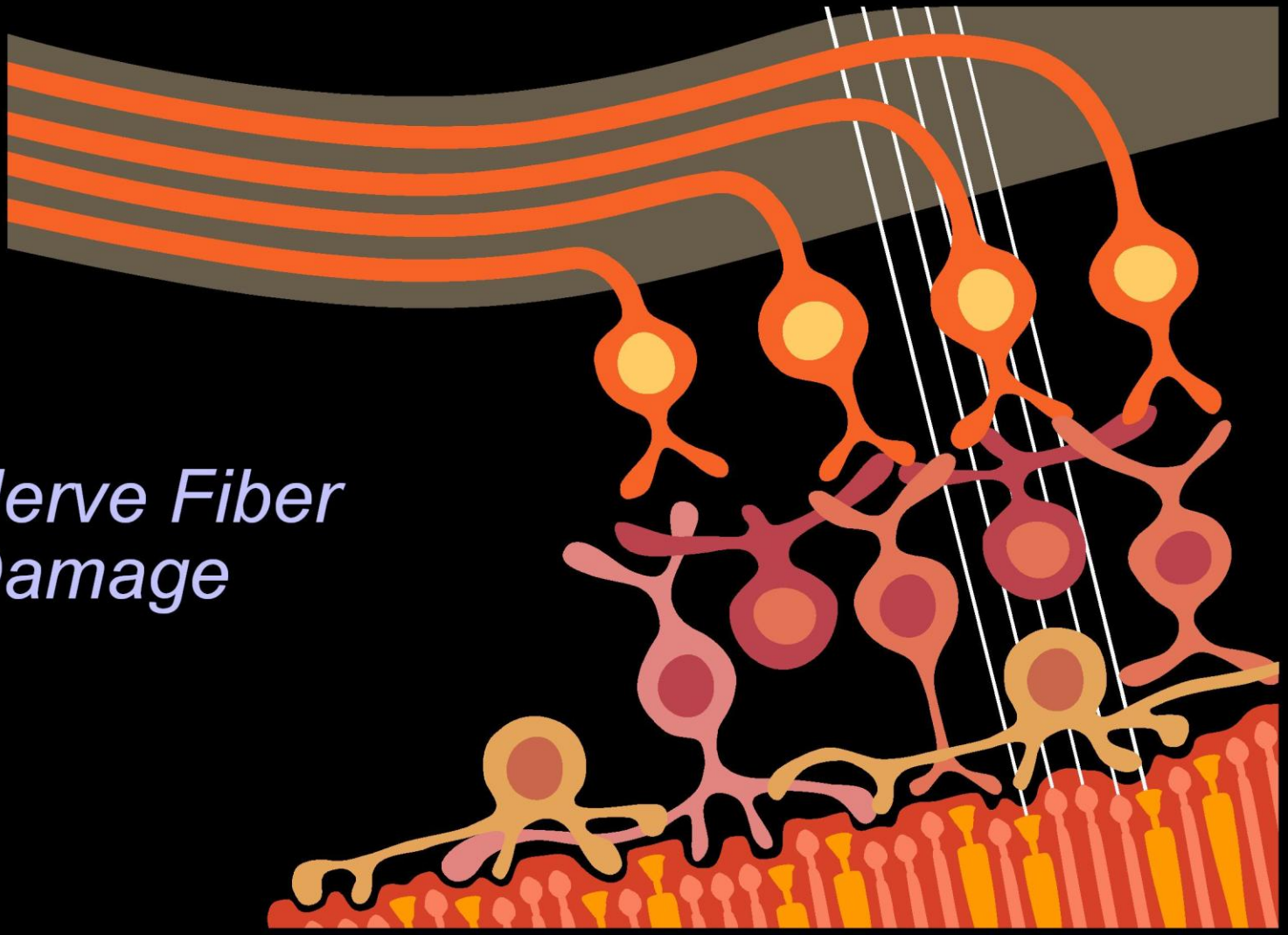
Primary Visual Cortex



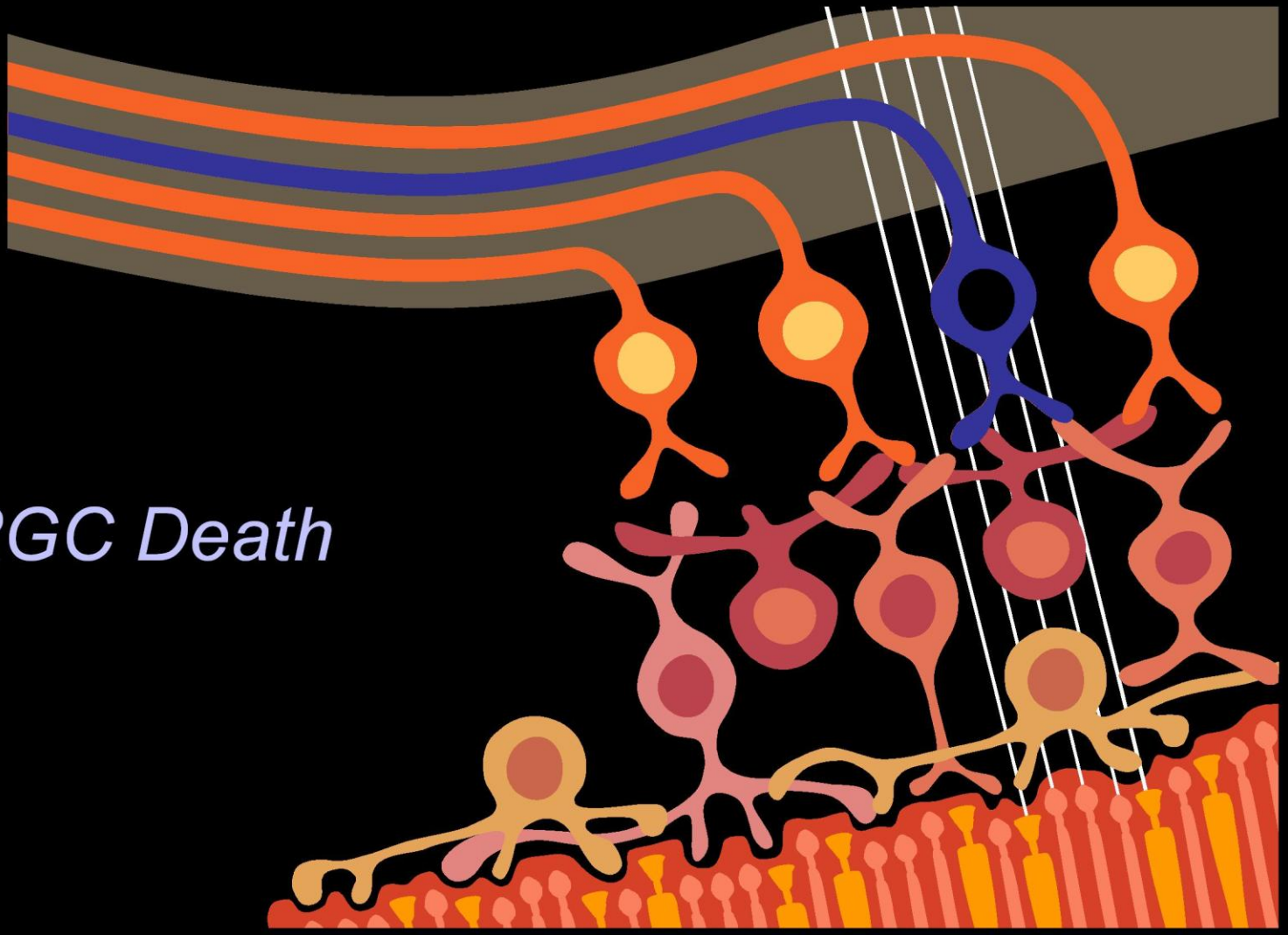
The Visual Pathway



Retinal Ganglion Cell Death

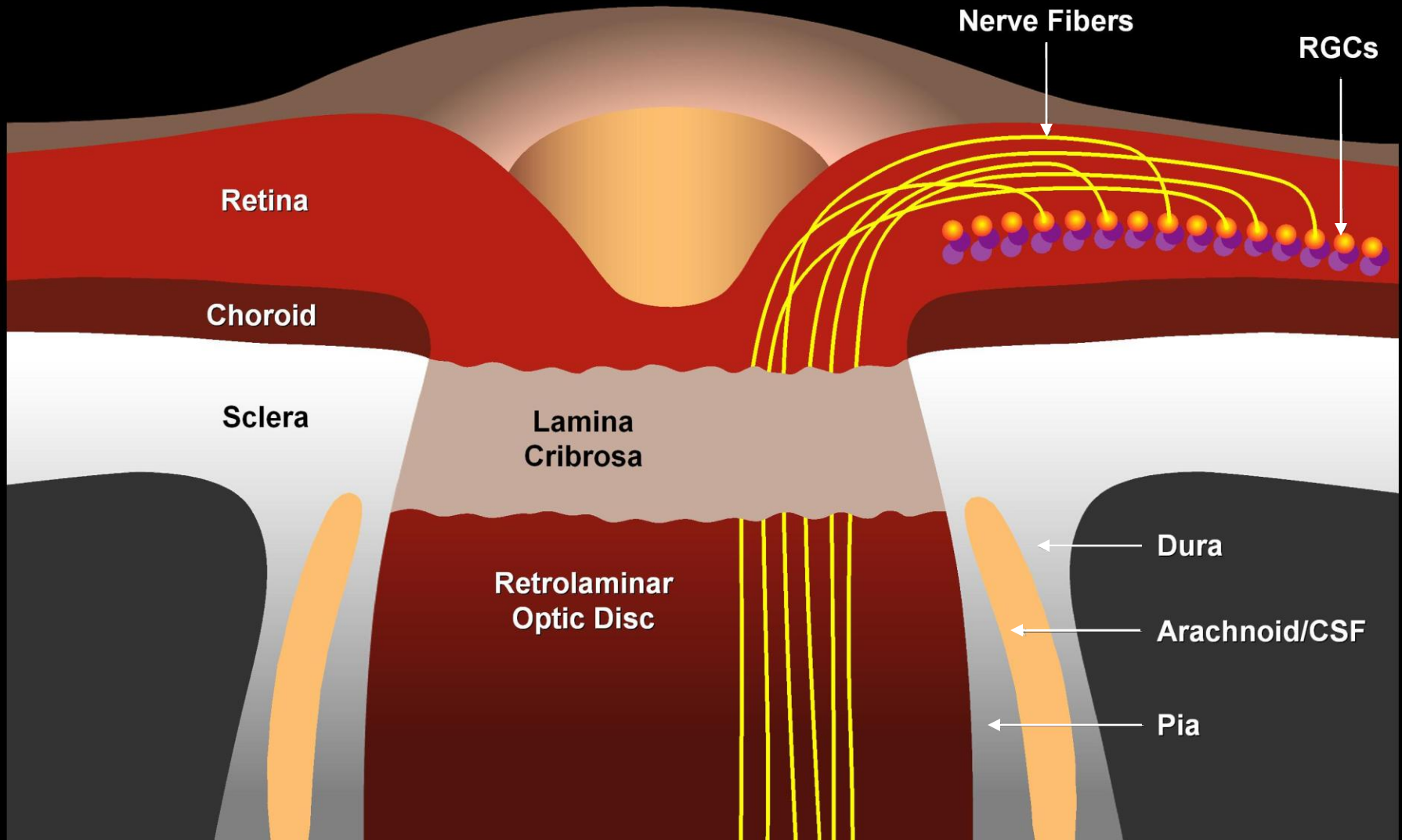


Retinal Ganglion Cell Death

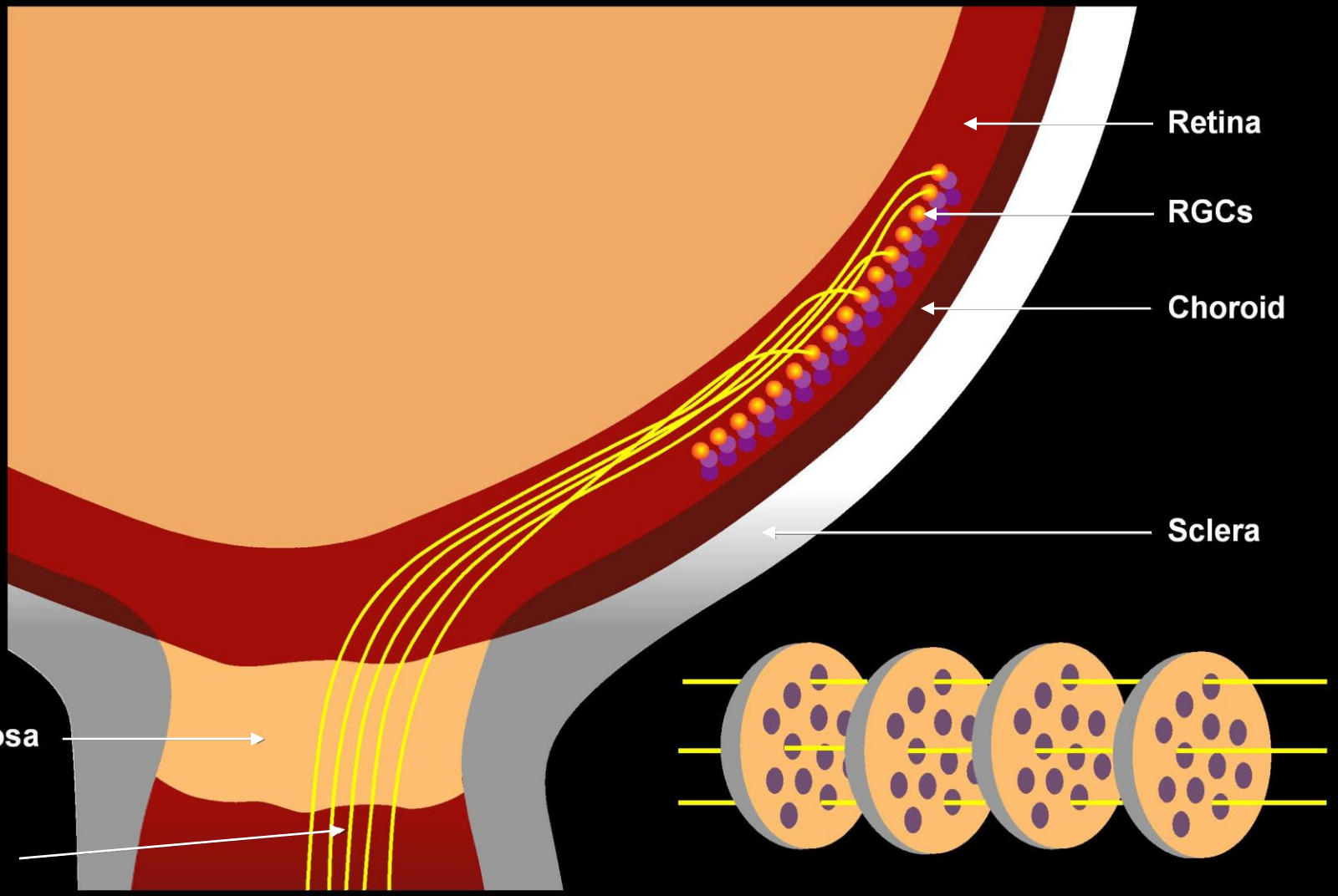


RGC Death

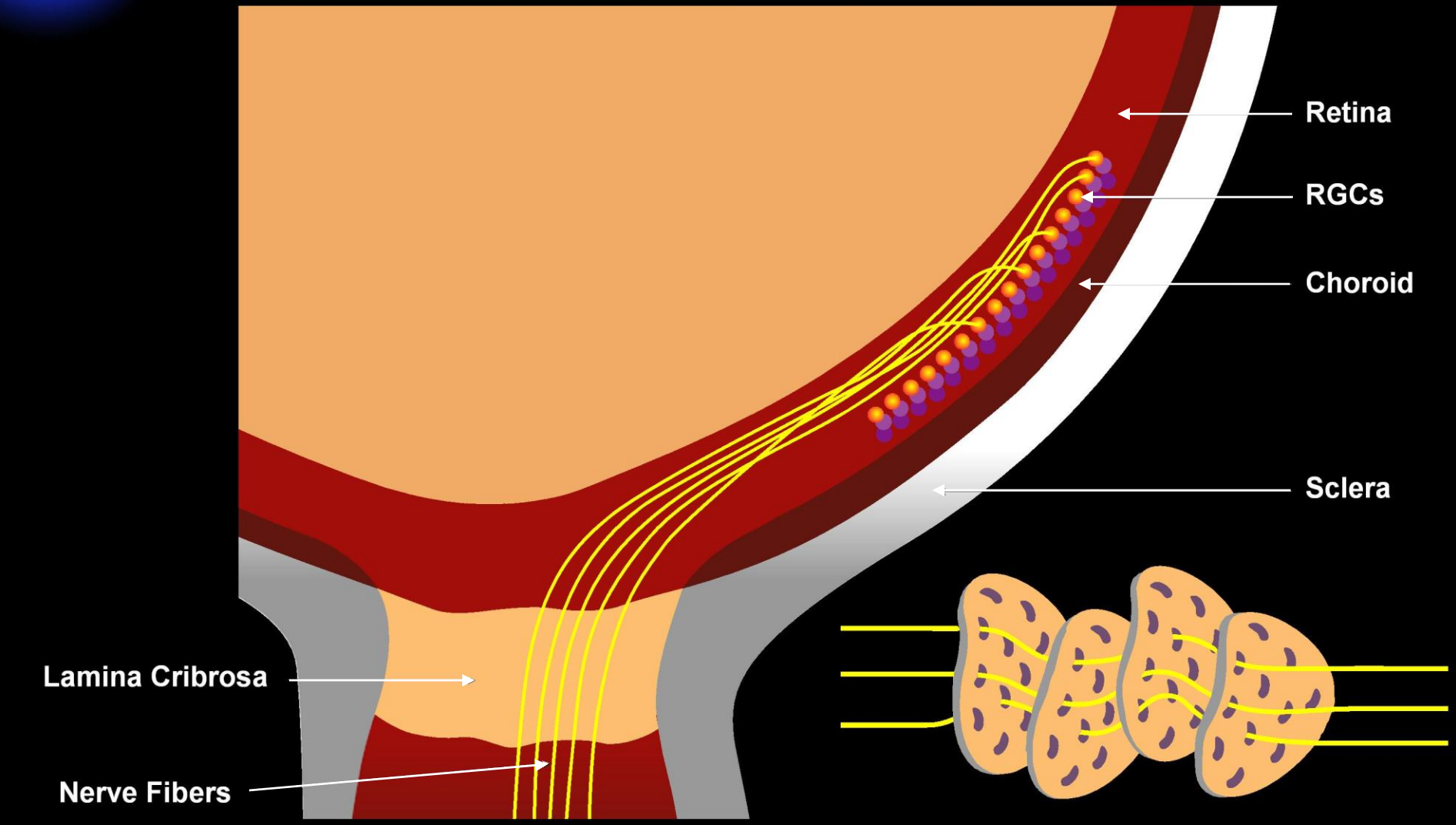
Glaucomatous Changes at the Optic Disc Level



Glaucomatous Changes in the Lamina Cribrosa

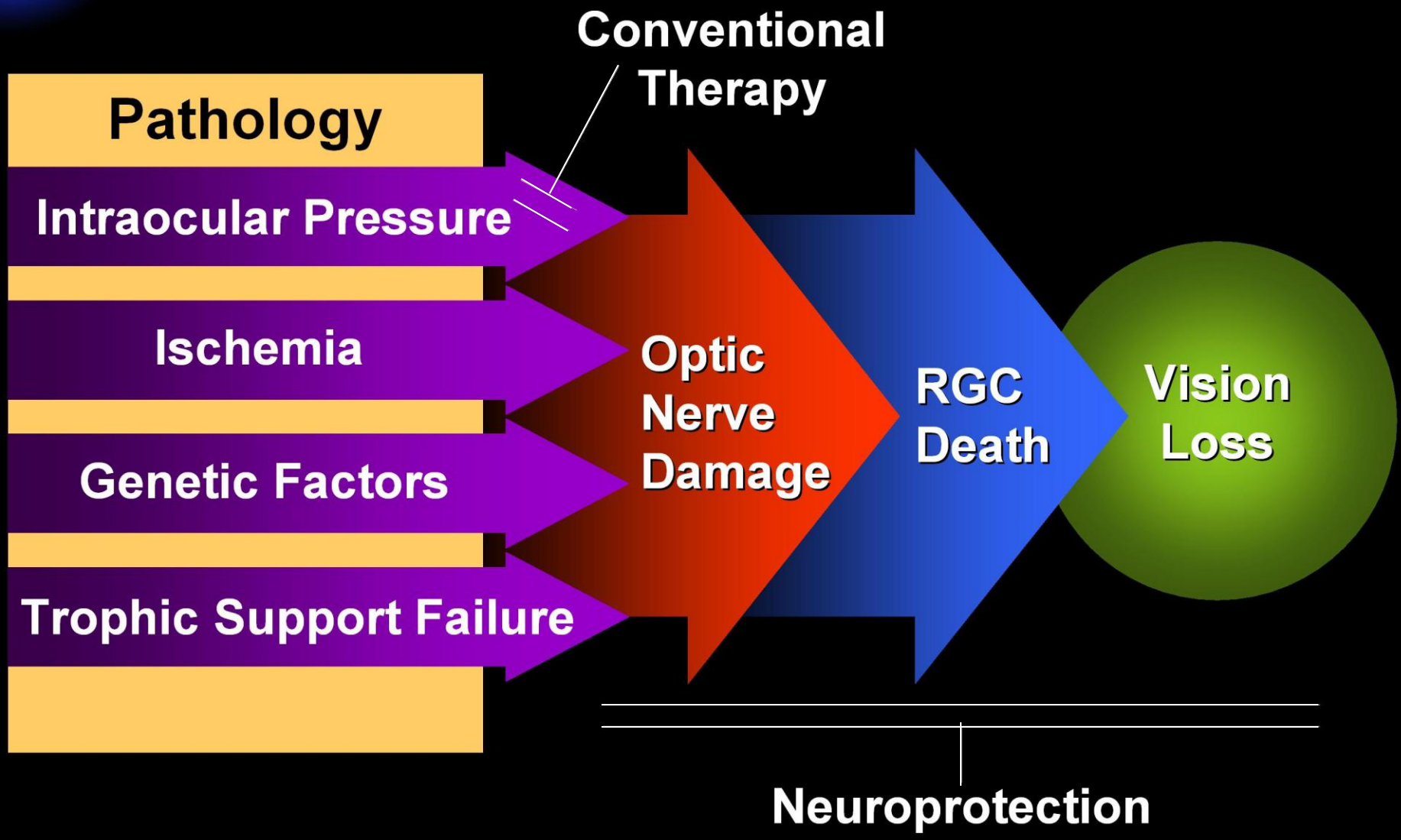


Glaucomatous Changes in the Lamina Cribrosa



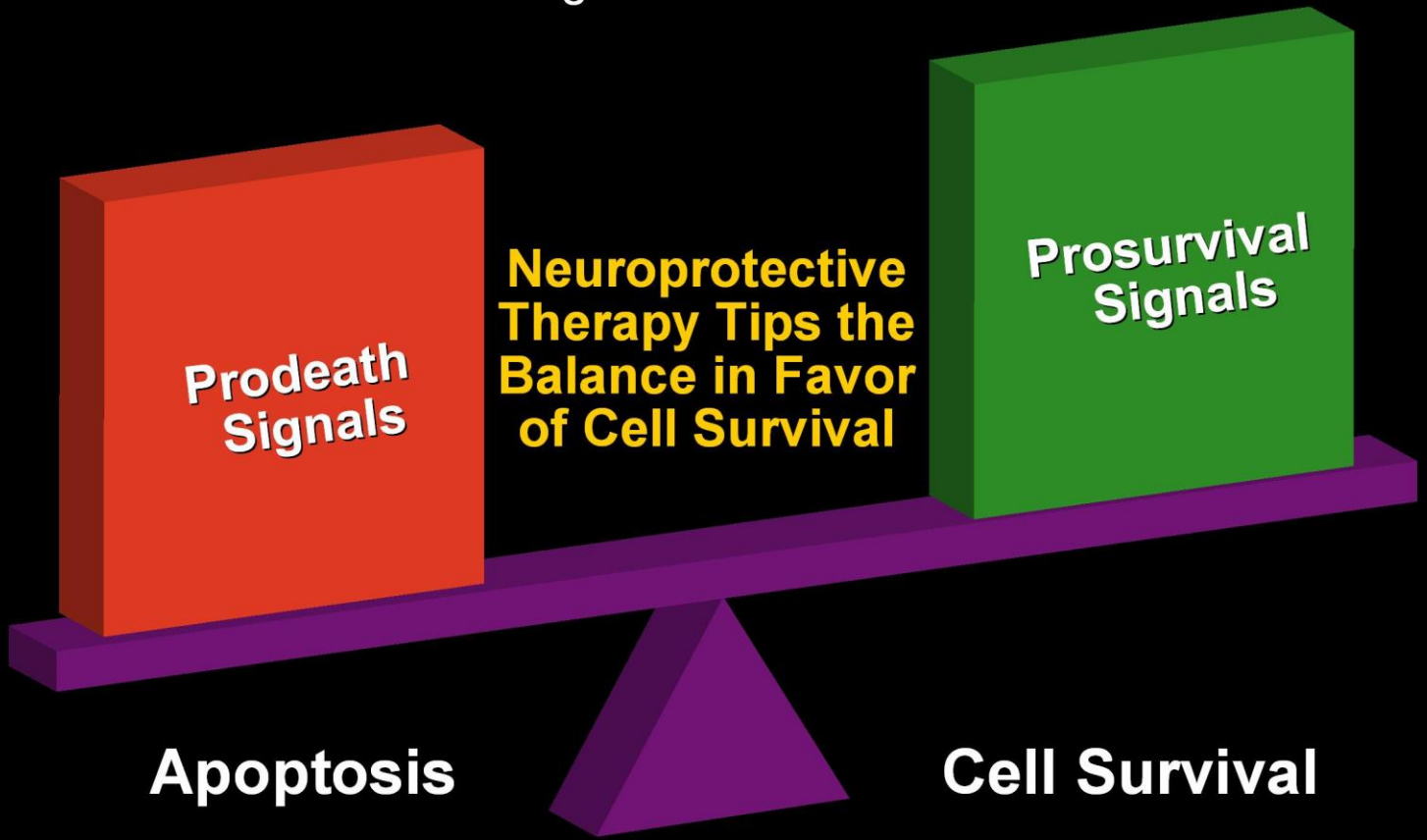
Mechanisms of Retinal Ganglion Cell Death in Glaucoma

Causes of RGC Injury

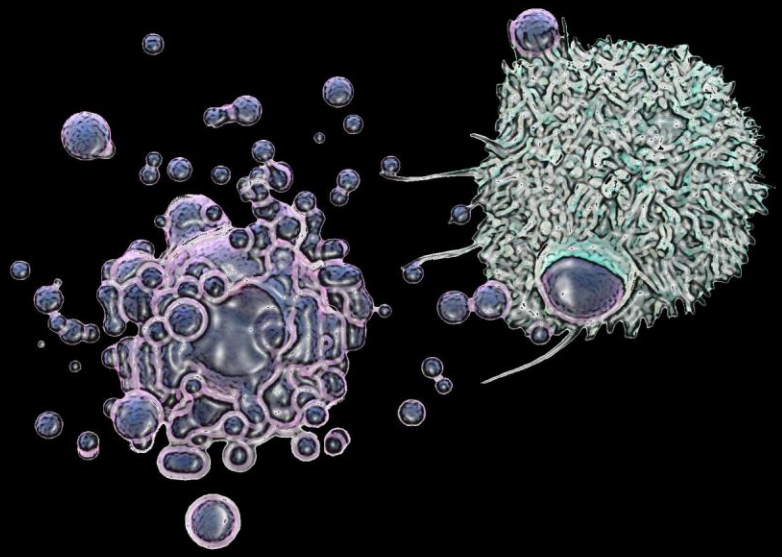


Stopping RGC Death Is a Balancing Act

- In normal eyes, various factors promote either the survival or death of RGCs
- RGC viability depends on balance between cell survival and death signals
- Goal of neuroprotective therapy is to tip balance in favor of cell survival
  - Block cell death signals
  - Enhance cell survival signals



Apoptosis: Cell Suicide



- Normal process of genetically programmed cell death that destroys cells that are injured or unneeded
- Apoptotic cell morphology:
  - Shrinkage of cellular nucleus and cytoplasm
  - Chromatin condensation
  - Formation of apoptotic bodies
  - Internucleosomal DNA fragmentation.

Apoptosis: Cell Suicide

- Debris from apoptotic cells is eliminated through phagocytosis
  - No inflammatory response
- Normally an important homeostatic function
  - But excessive or uncontrolled apoptosis is implicated in the pathogenesis of many diseases, including glaucomatous optic neuropathy

Why Target Apoptosis?

- Biochemical factors can increase RGC survival
- Drugs targeting these factors can be used to raise RGC resistance to cellular stress
- Upstream targeting does not stop apoptosis of severely damaged cells

Schuman. *Rev Ophthalmol*. 2001.

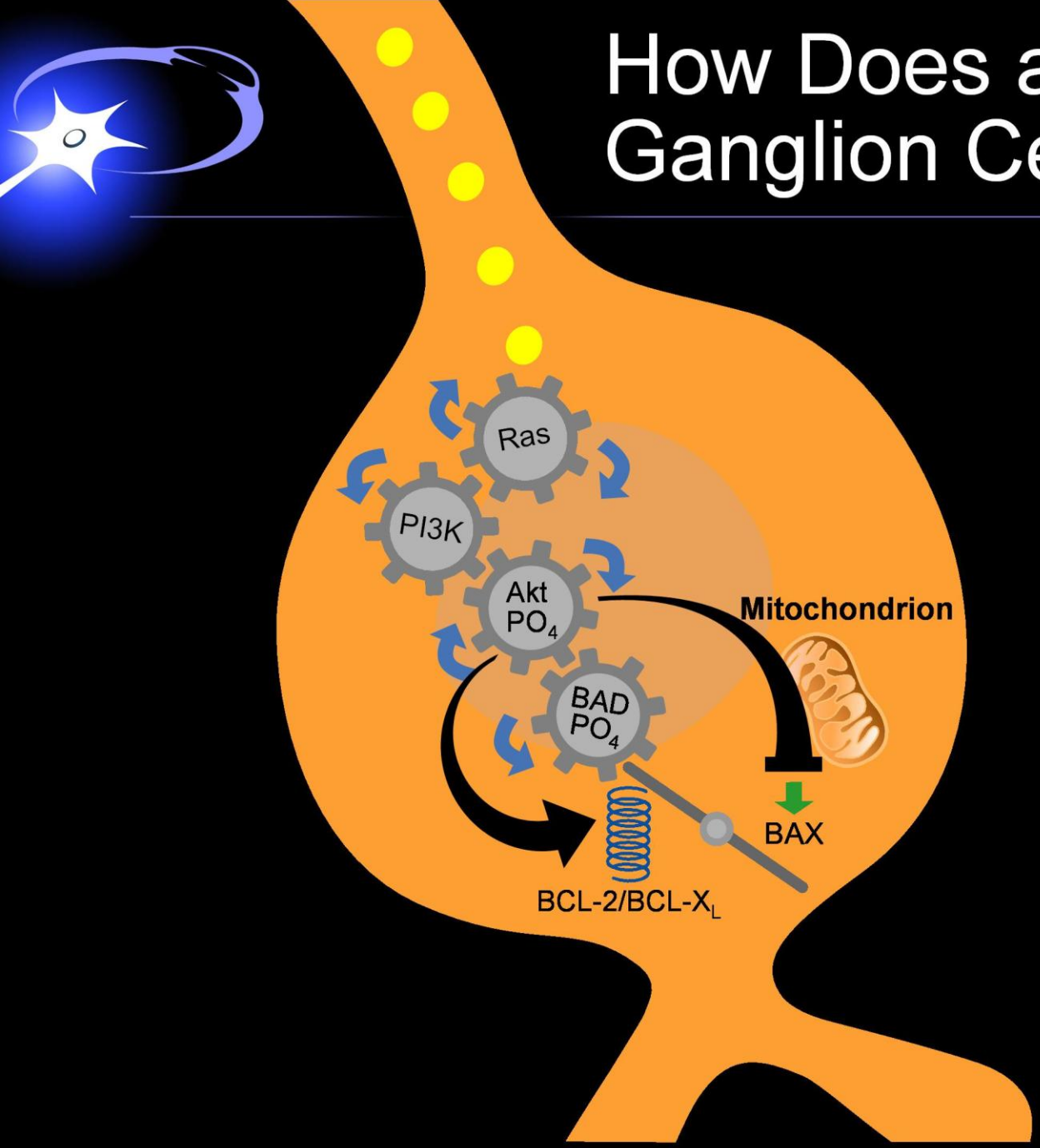
Apoptotic Signals

- Cell death signals include:
  - Glutamate excitotoxicity/NMDA receptor activation
  - Intracellular Ca++
  - Caspases
  - Mitochondrial cytochrome c leakage
  - Expression of apoptosis-promoting genes (eg, *bax*)
  - Underexpression of apoptosis-inhibitory genes (eg, *bcl-2, bcl-xL*)
  - Inflammatory cytokines (eg, TNFα, interleukins)
  - Nitric oxide and reactive oxygen species (free radicals)

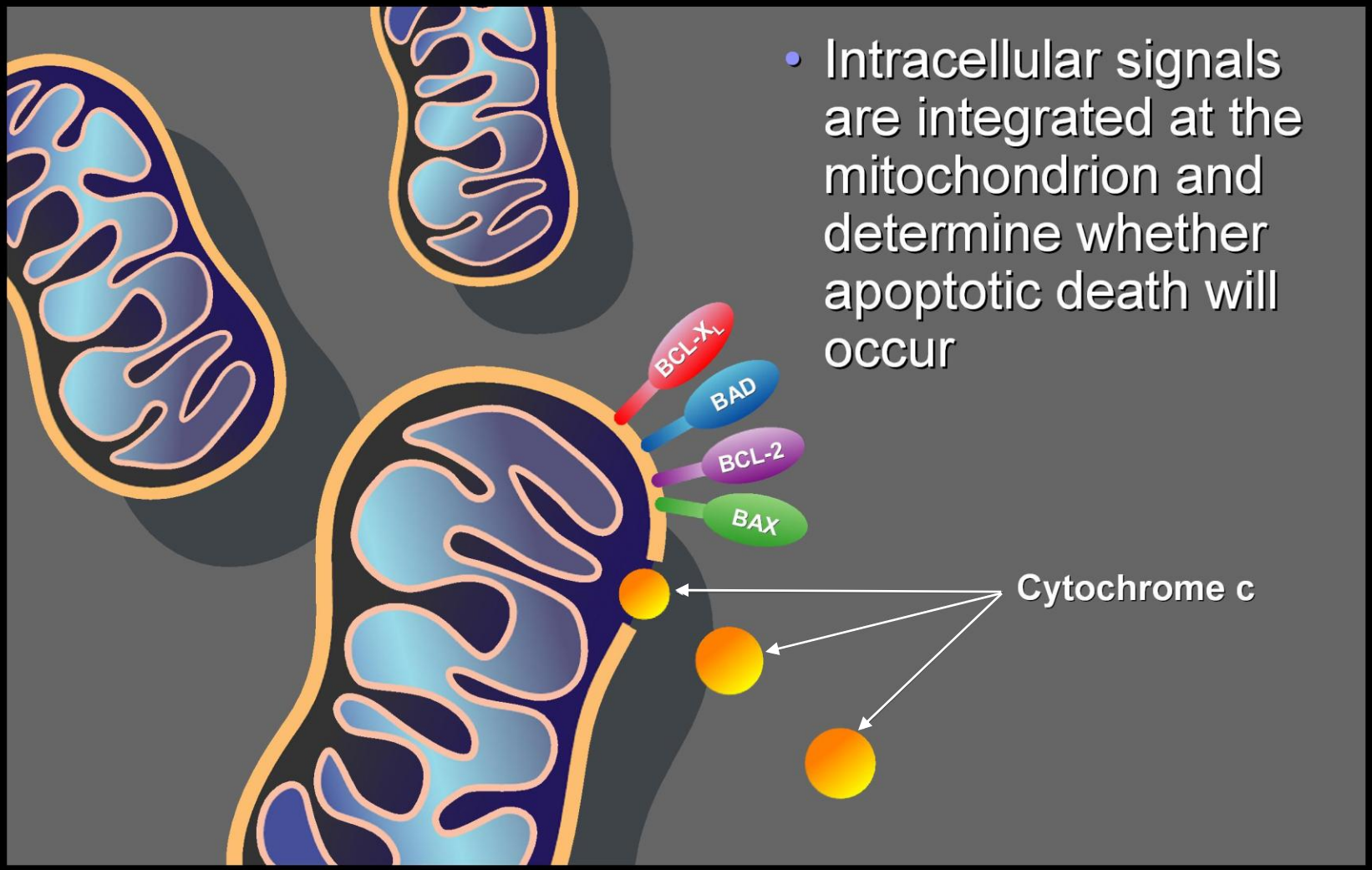
Cell Survival Signals

- Cell survival signals include:
  - Neurotrophins (growth factors)
    - eg, BDNF, bFGF
  - Expression of apoptosis-suppressing genes
    - eg, *bcl-2, bcl-xL*
  - Endogenous antioxidants
    - eg, glutathione, catalase, SOD
  - Adrenergic α_2-receptor–mediated pathways
    - eg, *bcl-2, bcl-xL*

How Does a Retinal Ganglion Cell Survive?

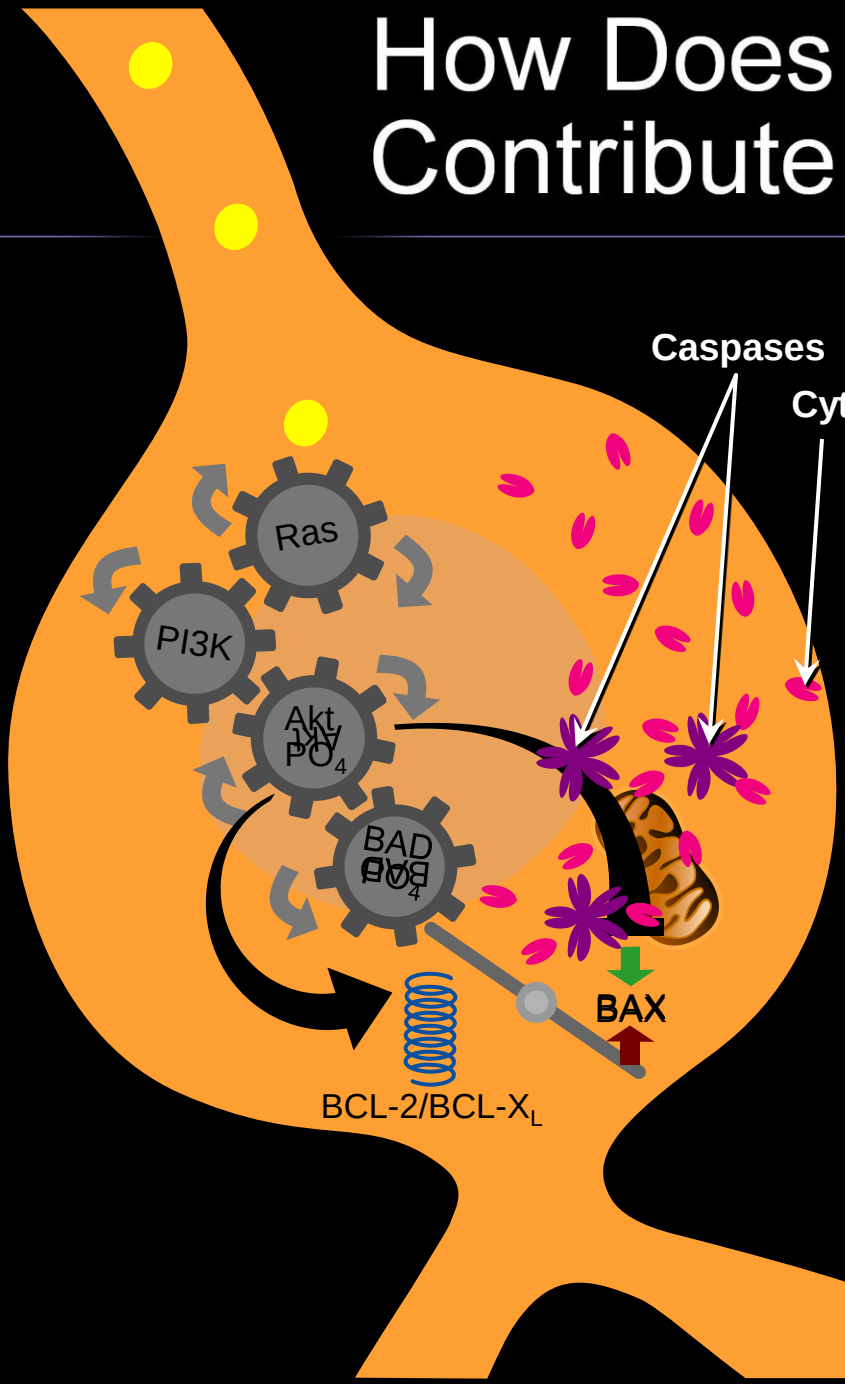


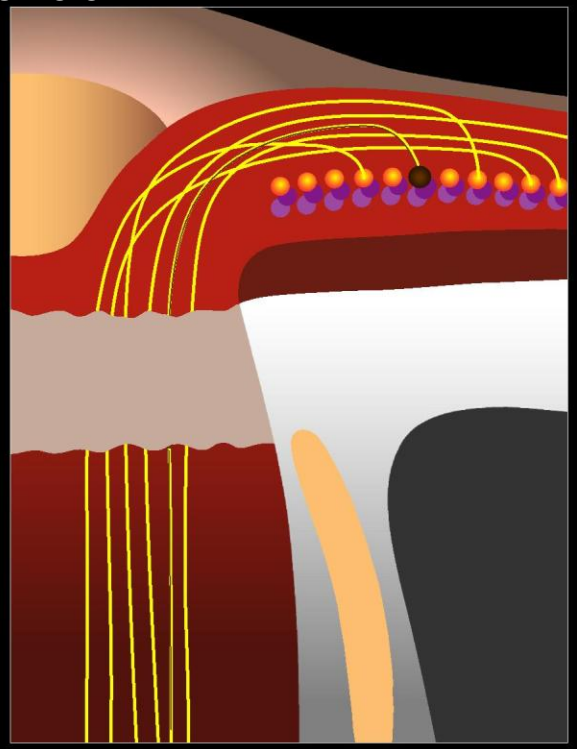
The Role of Mitochondria in Apoptosis



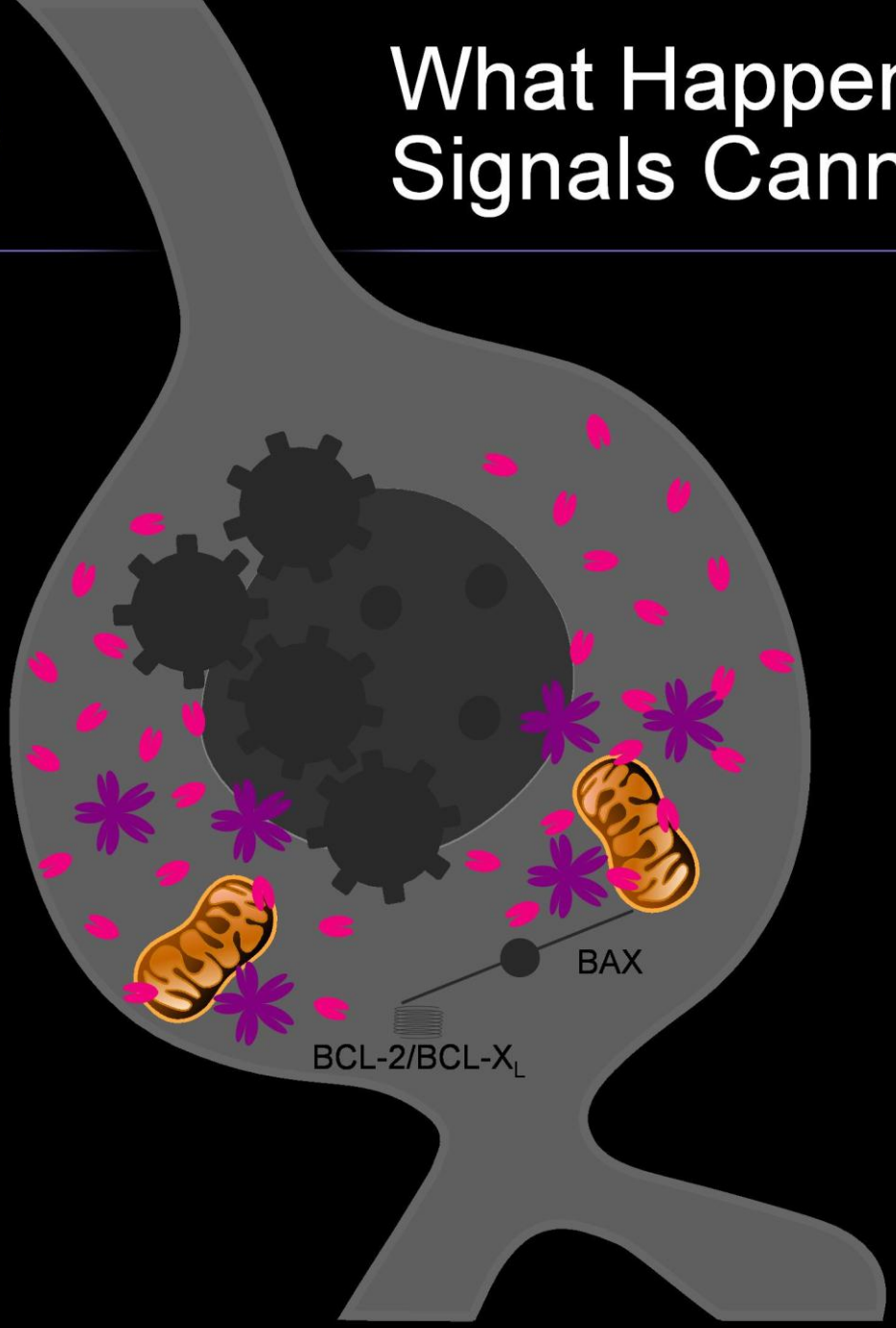
- Intracellular signals are integrated at the mitochondrion and determine whether apoptotic death will occur

How Does Elevated IOP Contribute to Apoptosis?

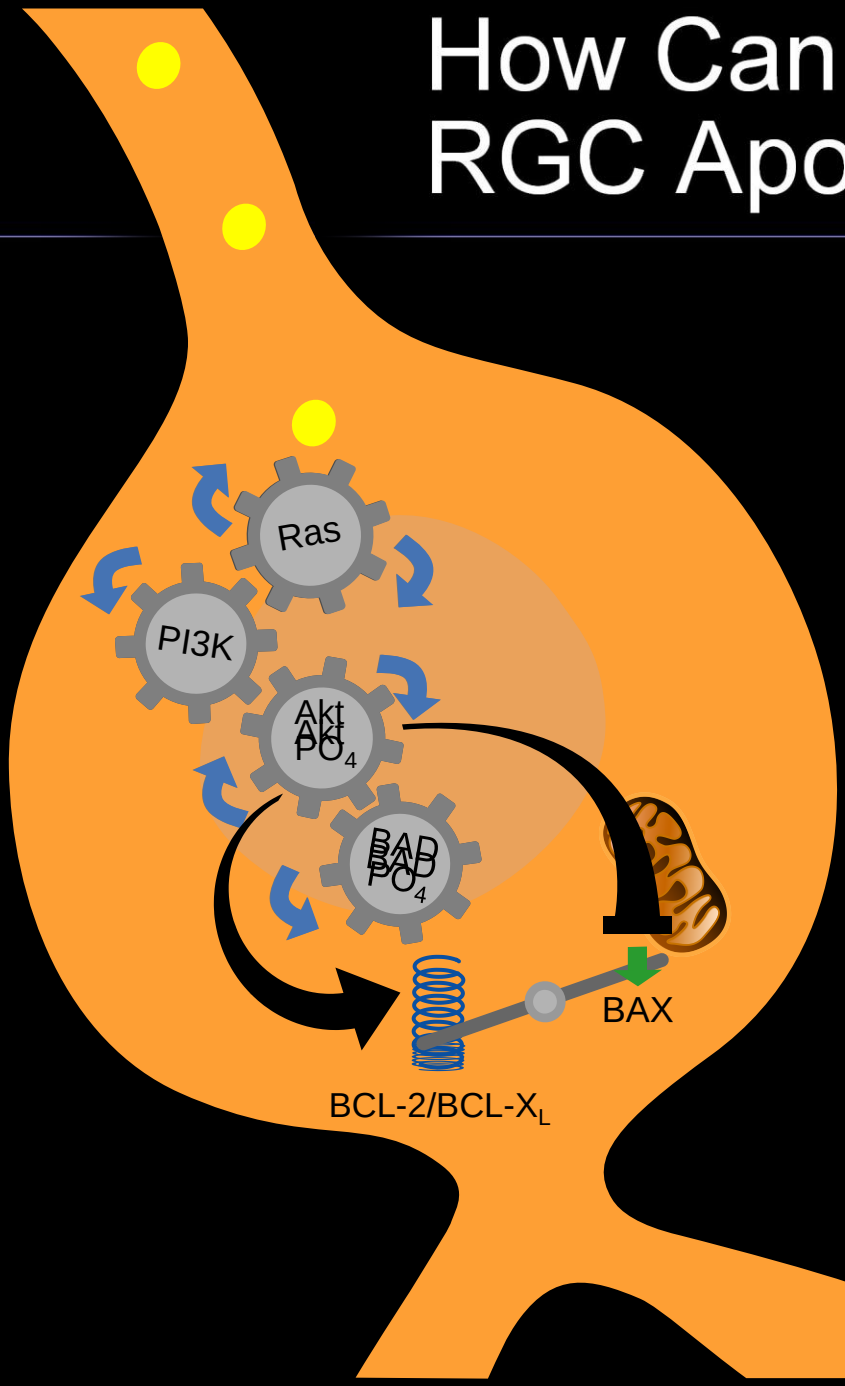


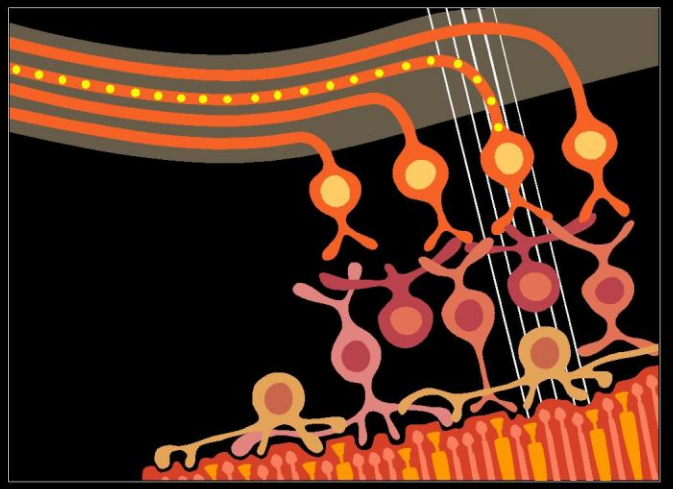


What Happens if Death Signals Cannot Be Stopped?

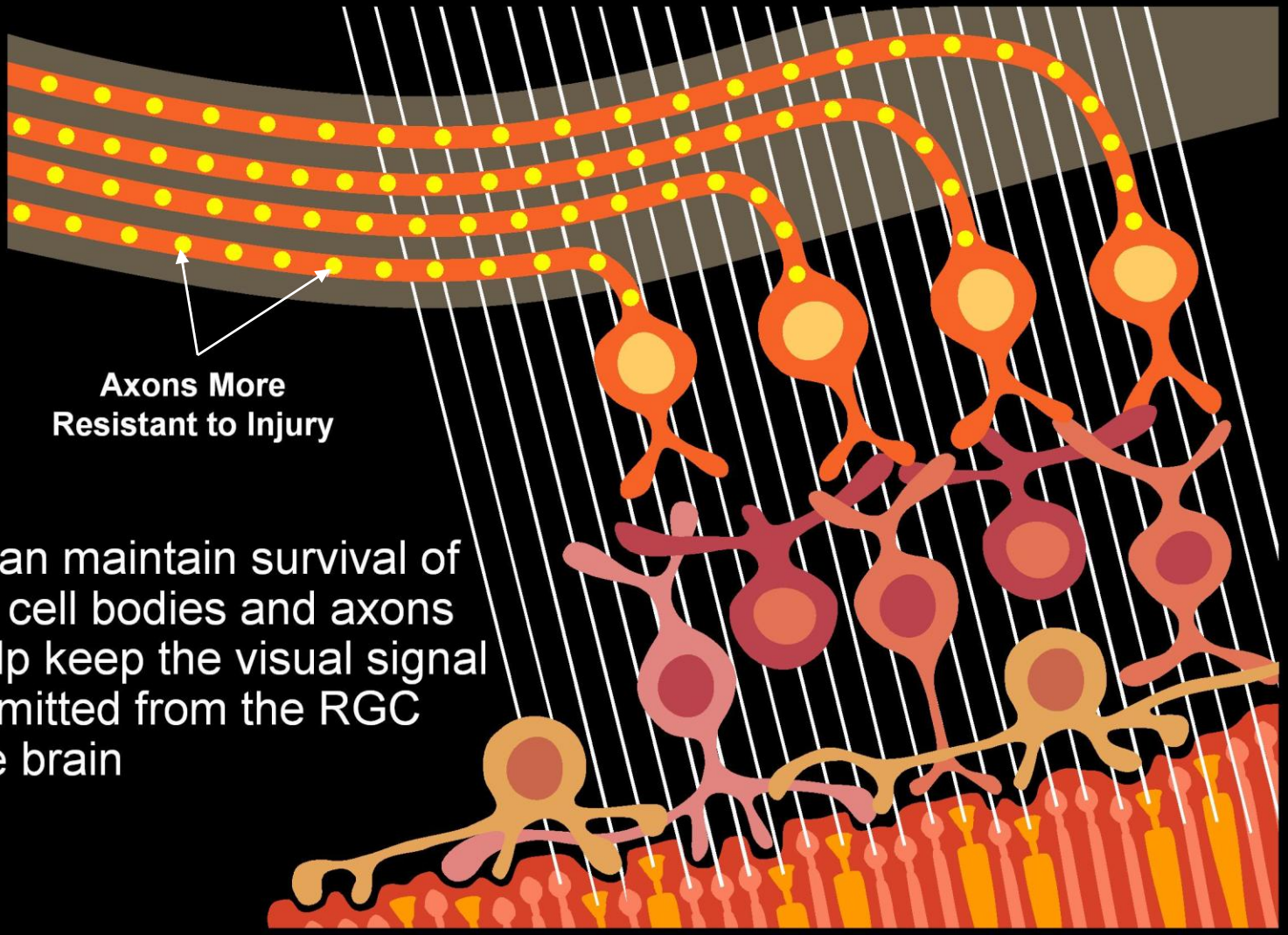


How Can We Stop RGC Apoptosis?





Promoting Cell Survival Preserves Structure and Function



- We can maintain survival of RGC cell bodies and axons to help keep the visual signal transmitted from the RGC to the brain

Glutamate and NMDA Receptor–Mediated Excitotoxicity

- May be important in mediating RGC injury
- Neuronal injury associated with other neurological disorders
  - Stroke and ALS as well as Alzheimer, Parkinson, and Huntington diseases

Lipton and Rosenberg. *N Engl J Med.* 1994.

Glutamate and NMDA Receptor–Mediated Excitotoxicity

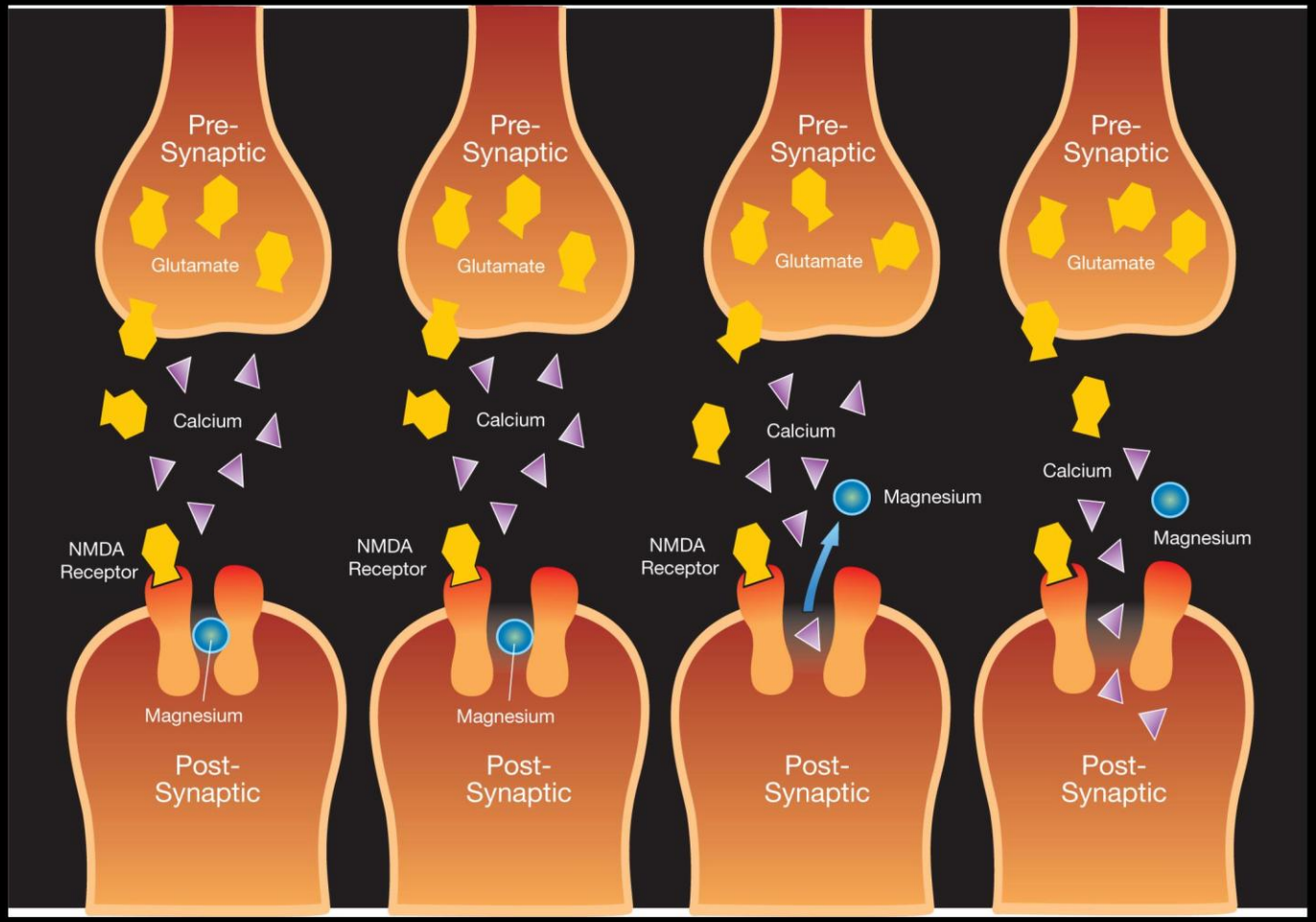
- 3 major subtypes of ionotrophic glutamate receptors: NMDA, AMPA, and kainate (KA).
- RGCs express both NMDA and non-NMDA–type glutamate receptors
- Overactivation of either subtype can produce excitotoxic injury
  - Excessive activation of NMDA-type receptors is especially deleterious
  - Results in increased Ca^{++} permeability and Ca^{++} influx → toxic to cells

Massey and Miller. *Neurophysiol.* 1990; Aizenman et al. *J Physiol.* 1988; Brandstatter et al. *Eur J Neurosci.* 1994; MacDermott et al. *Nature.*1986; Jahr and Stevens. *Nature.* 1987; Jahr and Stevens. *Proc Natl Acad Sci USA.* 1993.

Glutamate and NMDA Receptor–Mediated Excitotoxicity

- Excitoxicity hypothesis: glutamate is neurotoxic
- Principal excitatory retinal neurotransmitter
  - Activates several receptor subtypes within the retina
  - Activation of any of these receptors can be injurious to RGCS but activation of the NMDA receptor is particularly injurious
- Excessive glutamate may be toxic to normal RGCs through overstimulation of NMDA receptors
- Normal levels of glutamate can kill injured RGCs

Glutamate and NMDA Receptor–Mediated Excitotoxicity



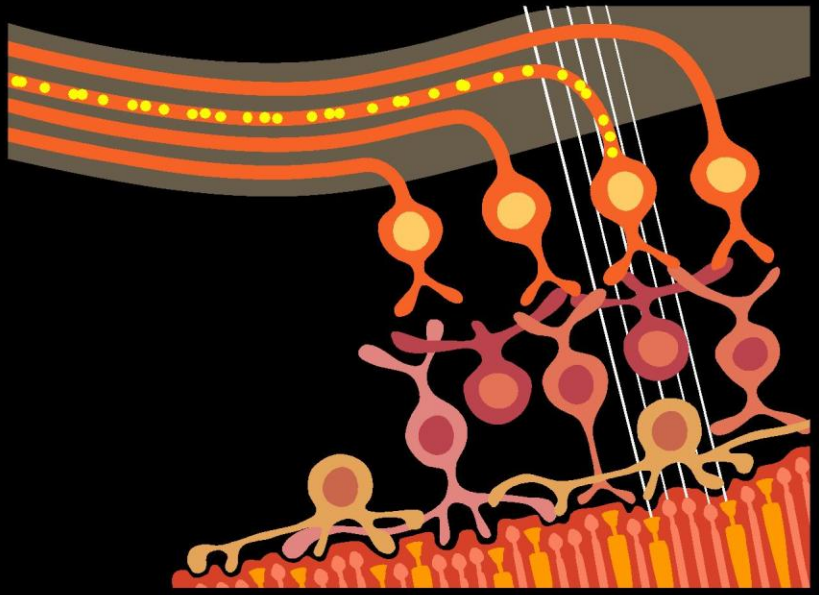
Glutamate and NMDA Receptor–Mediated Excitotoxicity

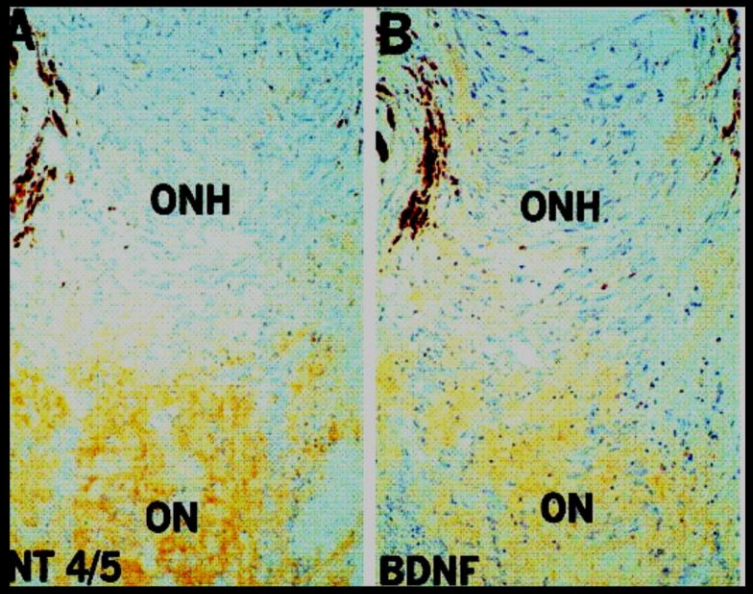
- NMDA-type glutamatergic toxicity suggested as mechanism for injury associated with ischemia and other neuronal insults (Watkins and Evans, 1981; Schumer and Podos, 1994)
- Search for NMDA antagonist therapies has increased in recent years
- Ample evidence that systemic glutamate antagonists can protect RGCs from neural injury (WoldeMussie et al, 2002; Hare et al, 2001, 2004a, 2004b; Gu et al, 2000)

Watkins and Evans. *Annu Rev Pharmacol Toxicol*. 1981; Schumer and Podos. *Arch Ophthalmol*. 1994; WoldeMussie et al. *J Glaucoma*. 2002; Hare et al, *Surv Ophthalmol*. 2001; Hare et al, *Invest Ophthalmol Vis Sci*. 2004a; Hare et al, *Invest Ophthalmol Vis Sci*. 2004b; Gu et al, *Nippon Ganka Gakkai Zasshi*. 2000.

What Is the Scientific Evidence That These Pathways Are a Realistic Description of What Happens in Glaucoma?

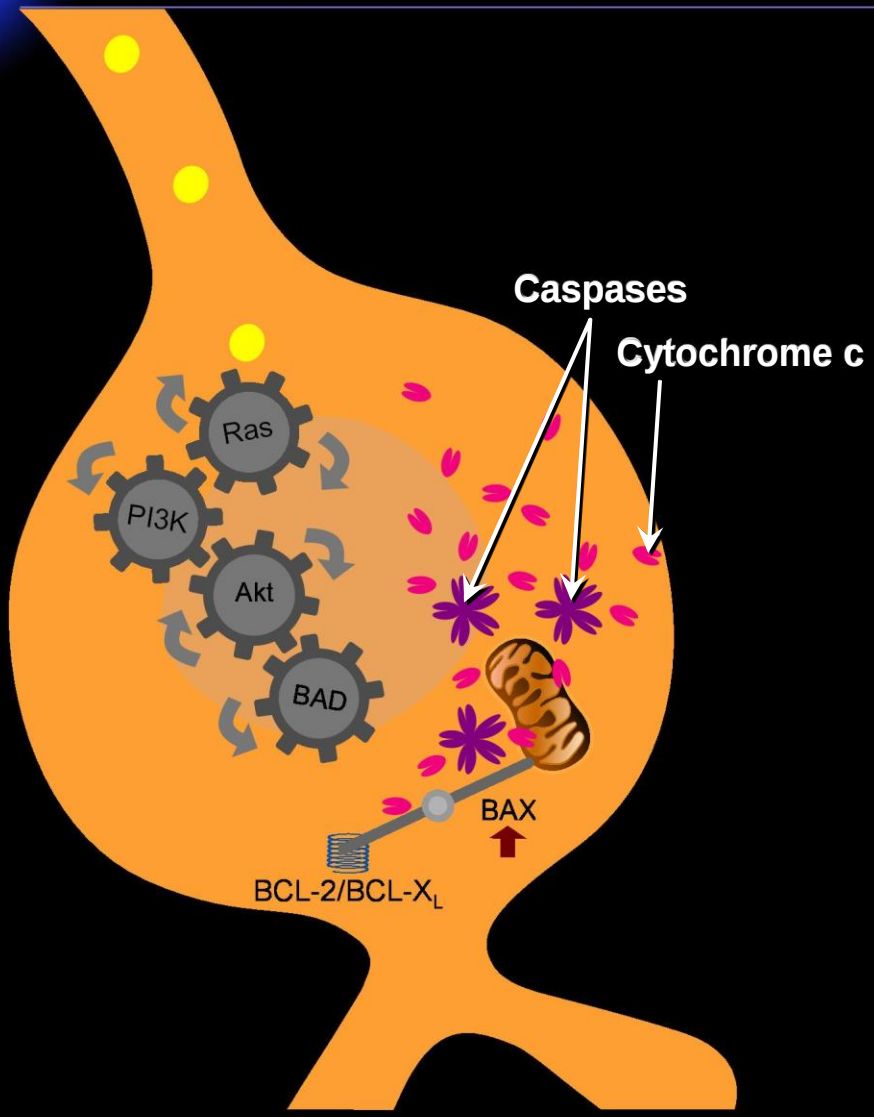
Axonal Transport Is Blocked in Glaucoma



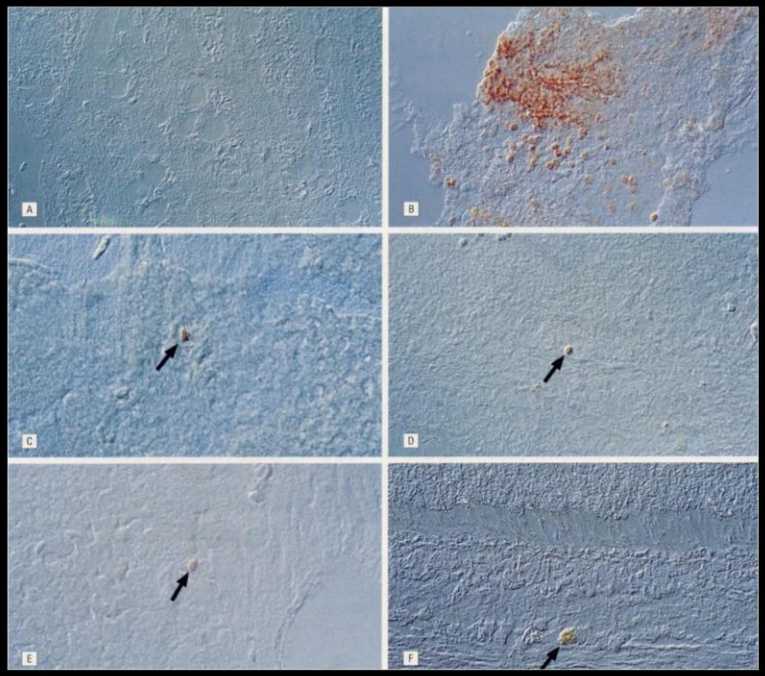


Johnson et al. *Invest Ophthalmol Vis Sci*. 2000.

Evidence for Apoptosis in Glaucoma

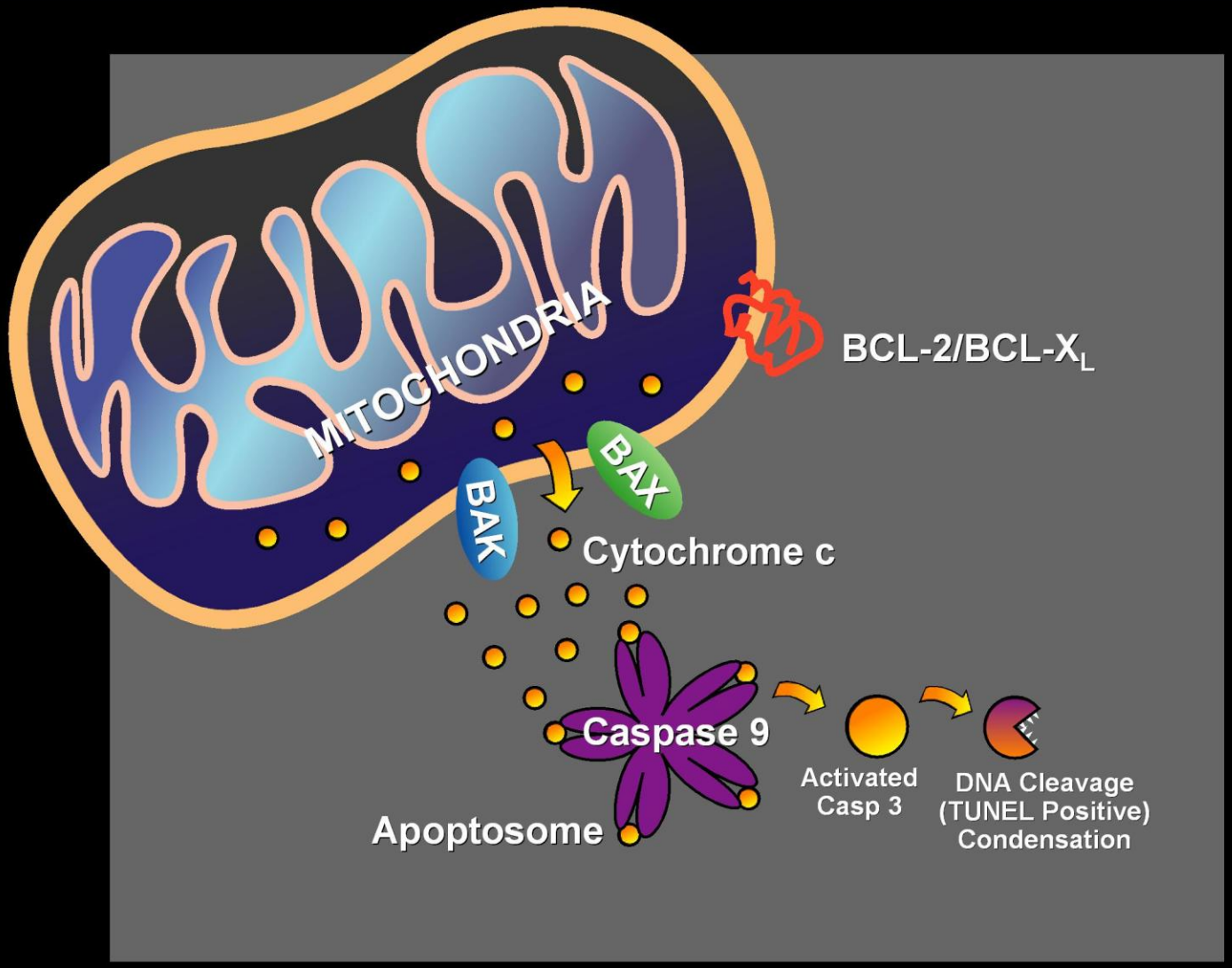


TUNEL staining of apoptotic RGCs in human glaucoma



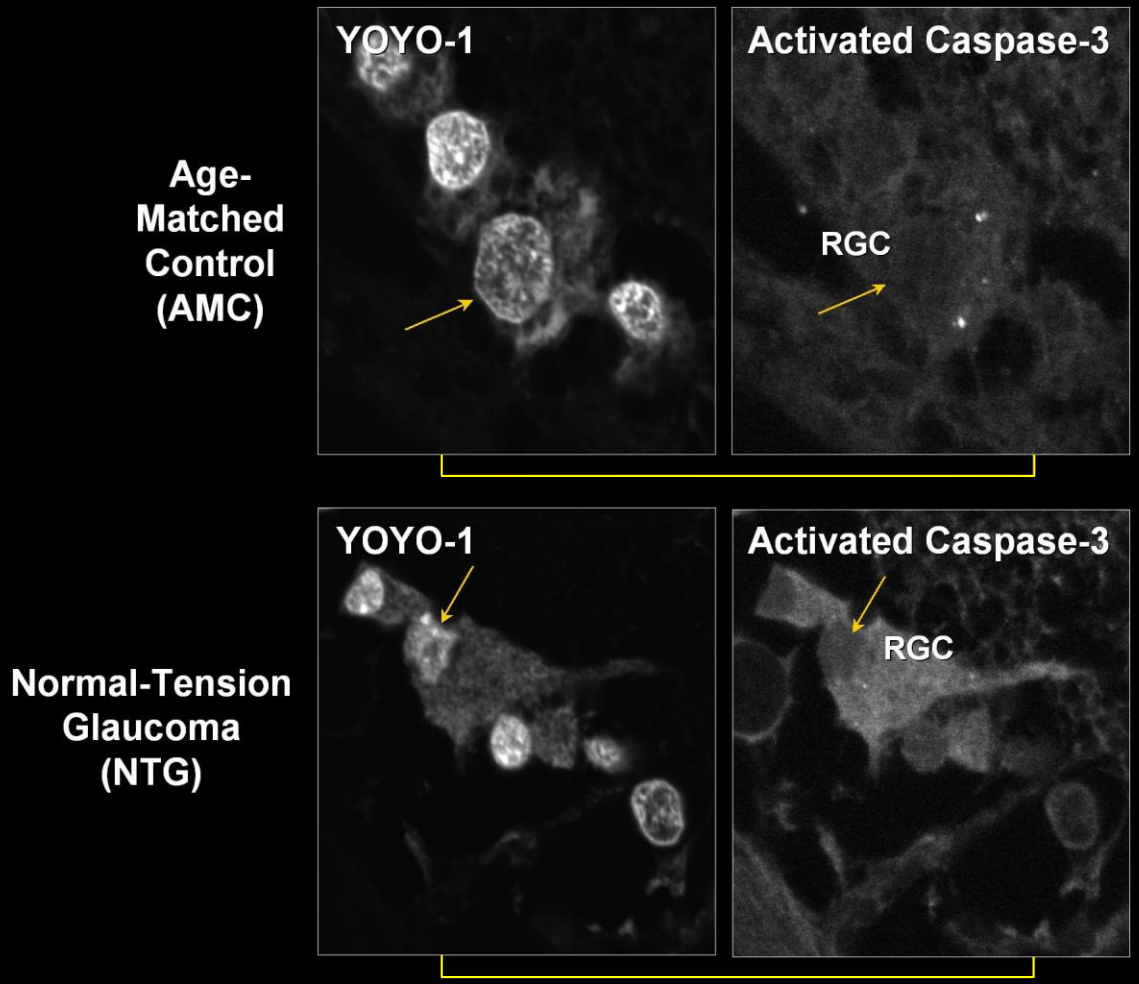
Kerrigan et al. *Arch Ophthalmol.* 1997.

Caspase Activation in Glaucoma



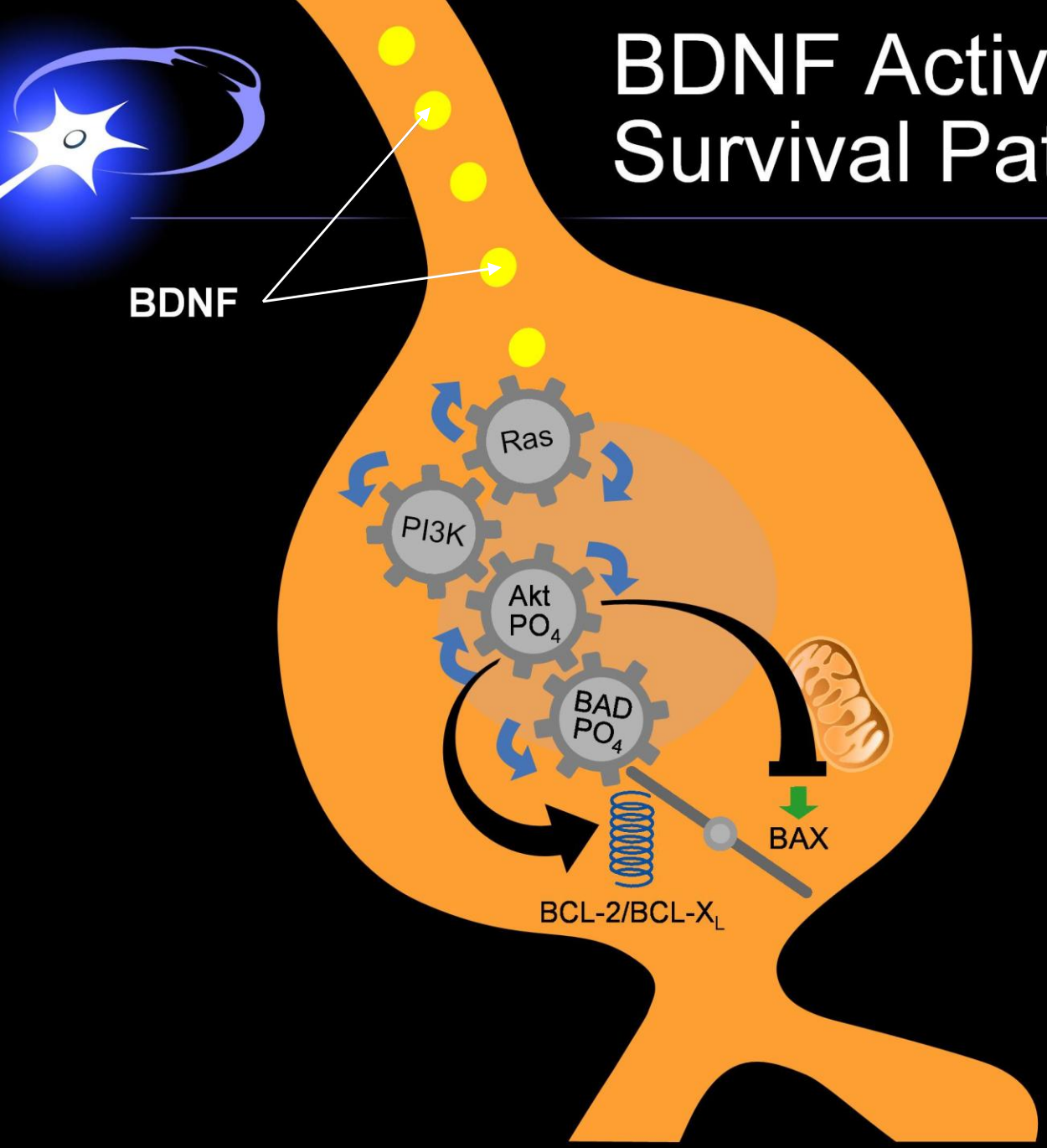
Tatton et al. *Ann Neurol*. 2003.

Growth Factor Insufficiency in Glaucoma Activates Executioner Caspases



Tatton, et al. *Surv Ophthalmol*. 2001.

BDNF Activates a Survival Pathway



Nakazawa et al.
Invest Ophthalmol Vis Sci. 2002.

Considerations in the Development of Neuroprotective Therapies

Rationale for Neuroprotective Strategy for Glaucoma

- Based on 3 critical observations:
  - Glaucomatous visual field loss in glaucoma is due to RGC death
  - Disease progresses with continual loss of RGCs
  - RGC loss may continue despite removal of the primary cause of injury

Rationale for Neuroprotective Strategy for Glaucoma

- Attempts to halt or minimize disease progression by stemming underlying process of neuronal injury and death
- Glutamate excitotoxicity, free radicals, and ultimately apoptotic cell death are involved in neurodegenerative processes
  - Neuroprotective strategies targeting these pathways are being investigated for the treatment of various diseases including glaucoma

Neuroprotection in Nonglaucomatous Diseases

- Once considered as treatment for neurological disorders
  - Head trauma, stroke, dementia, multiple sclerosis, ALS, and Alzheimer and Parkinson diseases
- Most therapies were not successful in clinical trials
  - Exceptions were neuroprotective therapies for Alzheimer disease, dementia, and spinal cord injury

Hickenbottom and Grotta. *Semin Neurol.* 1998;
Muir and Grosset. *Stroke.* 1999.

Why Could Neuroprotection Work in Glaucoma but not Other Diseases?

- Important pathogenic differences between glaucomatous and nonglaucomatous neurodegenerative diseases:
  - Glaucoma is a chronic, slowly progressing disease
    - Most unsuccessful therapies were aimed at acute patients
  - Location of the injury
    - The window of opportunity may be wider for preventing RGC death resulting from axonal injury than for cell body injury

Bracken et al. *JAMA*. 1997;
Weinreb and Levin. *Arch Ophthalmol*. 1999.

Experimental Neuroprotection Strategies

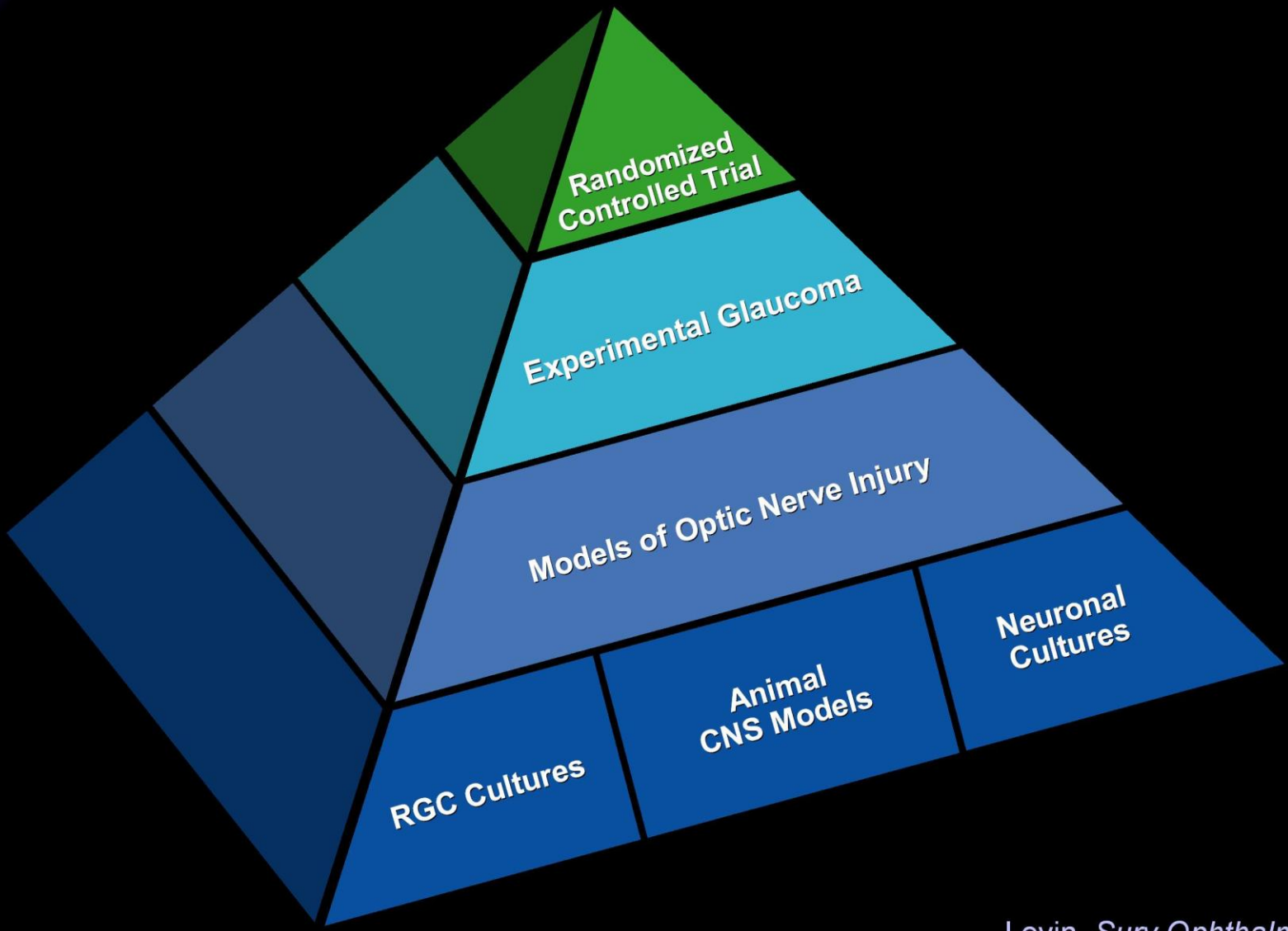
- Voltage-gated calcium channel blockade
- NMDA antagonism
- NOS inhibition/antioxidants
- Neurotrophin supplementation
- Caspase inhibition
- Apoptotic gene modulation
- Protease inhibition

Criteria for Evaluating Neuroprotective Agents for Glaucoma

1. Specific receptor target in the retina or optic nerve
2. Must reach the retina in pharmacologically effective concentrations
3. Activation of target must trigger pathways that enhance neuronal survival or decrease damage following optic nerve injury in animal models
4. Neuroprotective activity must be demonstrated in randomized, controlled, human clinical trials

Wheeler et al. *Surv Ophthalmol*. 2003; Levin. *Surv Ophthalmol*. 2003.

Data Supporting Neuroprotection Therapy



Levin. *Surv Ophthalmol.* 2003.

Challenges in the Development of Neuroprotectants for Glaucoma

- Glaucoma progresses slowly and rate is variable
  - Well-controlled studies require long duration and many subjects to show significant effects

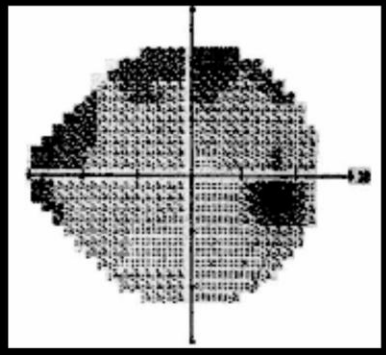
"Just as it has taken years to find neuroprotective treatments that work in most major neurologic diseases, it may be several years until clinical proof of neuroprotection in glaucoma and other optic neuropathies becomes available."

Francine Wein and Leonard Levin, 2002

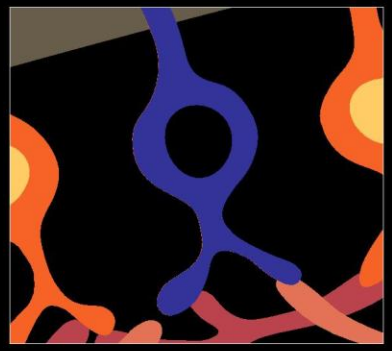
Wein and Levin. *Curr Opin Ophthalmol.* 2002.

Summary

- Glaucoma is an optic nerve disease in which patients can lose visual field despite IOP lowering



- RGCs die in glaucoma



- Preventing RGC death independent of IOP lowering is called neuroprotection