

Copyright Undertaking

This thesis is protected by copyright, with all rights reserved.

By reading and using the thesis, the reader understands and agrees to the following terms:

1. The reader will abide by the rules and legal ordinances governing copyright regarding the use of the thesis.
2. The reader will use the thesis for the purpose of research or private study only and not for distribution or further reproduction or any other purpose.
3. The reader agrees to indemnify and hold the University harmless from and against any loss, damage, cost, liability or expenses arising from copyright infringement or unauthorized usage.

IMPORTANT

If you have reasons to believe that any materials in this thesis are deemed not suitable to be distributed in this form, or a copyright owner having difficulty with the material being included in our database, please contact lbsys@polyu.edu.hk providing details. The Library will look into your claim and consider taking remedial action upon receipt of the written requests.

**PRESERVING VISION UNDER STRESS: RGC
VULNERABILITY TO ALKALI BURN AND
ELEVATED INTRAOCULAR PRESSURE**

QIU CHUNTING

PhD

The Hong Kong Polytechnic University

2026

The Hong Kong Polytechnic University
School of Optometry

**Preserving Vision under Stress: RGC Vulnerability to
Alkali Burn and Elevated Intraocular Pressure**

Qiu Chunting

A thesis submitted in partial fulfilment of the
requirements for the
degree of Doctor of Philosophy

October 2025

CERTIFICATE OF ORIGINALITY

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

_____ (Signed)

QIU Chunting
_____ (Name of student)

Dedication

To my parents, Mr. QIU Fengli and Mrs. YIN Xueqin, who gave me life, shaped me with love, and supported me at every moment of my journey.

This dissertation is dedicated to you.

Abstract

Purpose

Retinal ganglion cells (RGCs) serve as the final output neurons of the retina and as essential conduits for transmitting visual information to the brain. Given RGCs' inability to regenerate, any loss of RGCs results in permanent visual deficits, underscoring the need to understand how pathological insults compromise their survival to develop effective therapeutic strategies. This thesis investigates dysfunction and degeneration of alpha retinal ganglion cells (α RGCs) under two clinically essential conditions: chemical injury from corneal alkali burns and elevated intraocular pressure (IOP), which is relevant to glaucoma. Corneal alkali burn can cause irreversible damage to both the ocular anterior segment and posterior segment. Our first study aims to investigate the role of the gap junction subunit connexin 36 (Cx36) in mediating secondary cell death and its impact on the apoptosis of RGCs following ocular alkali burns. Since glaucoma is the most common cause of irreversible blindness, our second aim was to explore the effect on RGCs under different levels of high ocular hypertension and mechanical stress.

Methods

Wild-type C57BL/6J (WT) mice, KCNG-YFP, and homozygous connexin36-knockout (Cx36 KO HOMO) mice were used in the study. TUNEL staining and confocal microscopy were used to detect apoptotic cells. Intracellular dye injection was performed to confirm the neuronal morphology in the mouse retina. The light-evoked spikes, excitatory postsynaptic currents (EPSCs), and inhibitory postsynaptic currents (IPSCs) of RGCs were recorded using 525nm full-field light as the stimulus to assess the RGC function.

Study 1: Corneal alkali burn models were established in WT and Cx36 KO HOMO mice by applying 2 μ l of 1 M sodium hydroxide solution to the cornea. The gap junction blocker meclofenamic acid (MFA; 200 μ M) was administered via intravitreal injection immediately after the corneal alkali burn.

Study 2: Pressure-related injury was modeled using acute and sustained ocular hypertension to approximate glaucomatous stress. A sustained ocular hypertension (SOH) model was established in WT mice through 2 μ l silicone oil injection into the anterior chamber. An acute ocular hypertension (AOH) model was induced in WT mice by inserting a homemade micropipette connected to a saline-filled container into the anterior chamber for 50 minutes. The container height was calibrated to induce an intraocular pressure of 75 mmHg. Two direct mechanical pressure methods were applied to isolate immediate effects on RGC physiology: one using a pressure controller and another using direct homemade micropipette compression. Behavioral visual performance was evaluated using the optomotor response (OMR) assay, and retinal function was assessed with electroretinography (ERG).

Results

Study 1: Corneal alkali burn produced rapid damage in RGC number and function in WT mice. The RGC loss was characterized by time-dependent progression, peaking at 24 hours, spreading from peripheral to central retinal regions. Six hours post-injury was determined to be the appropriate time point to assess RGC functional deficits in the corneal alkali burn mouse model. Within 6 hours, transient α RGCs showed significantly reduced light sensitivity, EPSCs, and IPSCs (all $p < 0.05$). We identified Cx36 gap junctions as key mediators of secondary RGC death in this

setting. Both genetic ablation of Cx36 and pharmacological blockade with MFA significantly improved RGC survival after injury.

Study 2: In the SOH mice model, ERG recording showed significant decreases in positive scotopic threshold response (pSTR) and negative scotopic threshold response (nSTR), while there were no statistical changes in a-wave and b-wave after 4-week injury. The number of α RGCs was significantly reduced, whereas AII amacrine cell populations remained stable following 4-week injury. All types of α RGCs in 4-week SOH mice exhibited significantly reduced light sensitivities. In the AOH mice model, visual contrast sensitivity started to decline in 2 weeks but was significantly reduced by 4 weeks. There were significant decreases in α RGCs after 2-week and 4-week AOH injury, but no significant difference in morphology. All types of α RGCs in 4-week AOH mice exhibited significantly reduced light sensitivities. To test whether pressure directly perturbs RGC signaling, we applied controlled mechanical pressure to RGCs. Following sequential pressure application to α RGCs via a pressure controller, α RGC light sensitivity decreased. Moreover, micropipette-induced mechanical pressure decreased current amplitudes at various potentials without affecting reversal potentials.

Conclusions

This work elucidates how two major insults—corneal alkali burns and elevated IOP—compromise RGCs through distinct yet converging mechanisms. Following corneal alkali injury, RGCs underwent rapid early-stage decline involving Cx36 gap junction-mediated secondary death. The gap junction antagonist MFA demonstrated neuroprotective potential by blocking this secondary cell death process. Following IOP elevation, early behavioral deficits could be masked by compensatory mechanisms. However, damage to synaptic function and cell survival could be

detected, providing new insights for early diagnosis. Direct pressure manipulation experiments provide a mechanistic link between elevated IOP and visual dysfunction by revealing immediate reductions in light sensitivity and synaptic transmission, potentially due to spurious spiking and baseline fluctuations that mask genuine visual signals.

Publications

Qiu, C., Zhang, T., Wang, Q., Yang, K., So, C., & Pan, F. (2025). The Role of Connexin 36 Gap Junctions in Retinal Ganglion Cell Death After Corneal Alkali Burns. *Investigative Ophthalmology & Visual Science*, 66(12), 43. <https://doi.org/10.1167/iovs.66.12.43>

Conference Presentation

Qiu, C., & Pan, F. (2025). Reduced Light Sensitivity of Alpha Retinal Ganglion Cells in an Ocular Hypertension Mouse Model. *The Association for Research in Vision and Ophthalmology (ARVO)*. May 2025.

Qiu, C., Wang, Q., Yang, K., & Pan, F. (2024). The Involvement of Connexin 36 Gap Junctions in Retinal Ganglion Cell Death following Corneal Alkali Burns. *The Association for Research in Vision and Ophthalmology (ARVO)*. May 2024.

Acknowledgements

I would like to express my sincere gratitude to everyone who has been part of my PhD journey.

First and foremost, I am deeply grateful to my wonderful parents, Mr. Qiu and Mrs. Yin, the greatest in the world, as well as my other family members - my grandma, grandpa, uncle, and auntie. Under any circumstances, they have unconditionally encouraged, accompanied, and supported me. No matter what happens, they love me unconditionally, and I love them too.

I extend my heartfelt gratitude to The Hong Kong Polytechnic University and the School of Optometry for giving me the opportunity to pursue my studies here and for their invaluable guidance.

I would like to express my profound respect and appreciation to my supervisor, Prof. Pan Feng. His extensive knowledge, meticulous approach to scholarship, and commitment to lifelong learning are truly inspiring. His guidance and assistance in both my research and career development have been instrumental in shaping my academic growth. I wish him lasting joy and passion as he continues to excel and make significant contributions in his academic career.

I also wish to acknowledge my co-supervisor, Prof. Do Chi Wai, with deep gratitude for his professional academic counsel and ongoing support. His guidance has significantly expanded my academic horizons by offering fresh perspectives. Furthermore, I extend my appreciation to his students, Dr. Cui Yingkun, Ms. Li Huan, and Dr. Margaret, for their invaluable assistance and thoughtful recommendations throughout my studies.

I would also like to express my heartfelt appreciation to Prof. Zhou Lei for his expert academic mentorship and patience, along with the extensive help he has provided. I also extend my appreciation to his student, Ms. Erin, for her generous help.

I would also like to express my deep appreciation to Prof. Lin Bin, Prof Henry Chan, and Prof. Dennis Tse for their valuable contributions and encouragement throughout my studies. Additionally, I would like to thank the research team: Dr. Li Rong, Dr. Cheng Meng, Dr. Zhang Jing, Ms. Wu Jiangmei, Mr. Patrick, and Mr. Wang Jiajun, for their patience, generosity, guidance, and the expertise they shared during my academic journey.

I recognize the invaluable contributions and joint endeavors of all academic teams and research partners at PolyU, which formed the foundation for this investigation. I am particularly thankful to my laboratory associates, Dr. Seema Banerjee, Dr. Qin Wang, Dr. So Chunghim, Ms. Zhang Ting, and Ms. Yang KangYi, for their solidarity, mutual support, and shared commitment to excellence.

My sincere appreciation goes to my research companions and experienced colleagues who provided substantial guidance in my laboratory work and dissertation writing: King Kit Li, Christie Lam, Yang Xiaying, Lu Daqian, Liu Zhengji, Liang Yuanyuan, Liang Dong, Patience, Huang Yingying, and Kryshell.

Thanks to my besties, PAN Xinghui, who has been my companion through twenty years of friendship. Thank you for being by my side and supporting me through every stressful moment of this journey.

Thanks to my dearest friend, ZHANG Jing. Meeting her during my PhD has been one of my greatest blessings. She is an extraordinarily brilliant and resilient woman who has offered me not

only invaluable academic insights but also unwavering emotional support. My PhD journey would have been unimaginable without her friendship and strength.

Special thanks to my boyfriend, Mr. Wang Jiaqing, who has been by my side through every moment, both joyful and challenging, and has consistently provided me with emotional and academic support. Without him, I would not have been able to complete this journey. I extend my gratitude to my uncle and auntie for inspiring me in all my pursuits and for their steadfast support throughout.

I appreciate everyone who has shared in this journey with me.

Contents

Chapter1 Introduction	19
1.1 The anatomy of the eye.....	19
1.1.1 Cornea.....	20
1.1.2 Retina.....	20
1.2 Retina structure and neurons.....	21
1.2.1 Photoreceptors	23
1.2.1.1 Rod and cone cells	23
1.2.1.2 Rod and cone pathway	26
1.2.2 Bipolar cell.....	30
1.2.3 Horizontal cell.....	33
1.2.4 Amacrine cell	35
1.2.4.1 AII amacrine cell.....	36
1.2.4.2 Dopaminergic amacrine cell	38
1.2.5 Retinal ganglion cell	40
1.2.5.1 Classification of retinal ganglion cells.....	40
1.2.5.2 Alpha retinal ganglion cells	44
1.2.5.2.1 Inputs of α RGCs	46
1.2.5.2.2 Outputs of α RGCs.....	47
1.3 Synapse in the retina	49
1.3.1 Chemical synapse	49
1.3.2 Electrical synapses.....	50
1.3.2.1 Gap junction structure, function, and regulation	50
1.3.2.2 Neuronal gap junctions in the retina	54
1.3.2.3 Connexin36.....	57
1.3.2.1.1 Connexin36 involved in the visual pathway.....	57
1.3.2.1.2 Connexin36 involved in secondary retinal cell death.....	60

1.4 Ocular alkali burn	62
1.4.1 Pathophysiology and mechanism of anterior segment damage after ocular alkali burn	62
1.4.2 Pathophysiology and mechanism of posterior segment damage after ocular alkali burn	65
1.4.3 Cell apoptosis.....	66
1.4.3.1 Morphology and significance of cell apoptosis	66
1.4.3.2 Role of TNF- α in the cell apoptosis pathway	67
1.4.4 Apoptosis of retinal ganglion cells in the ocular alkali burn	68
1.4.4.1 The changes of TNF- α in neurodegenerative diseases.....	68
1.4.4.2 The generation of TNF- α from microglia	69
1.4.4.3 Therapeutic option for retinal ganglion cell apoptosis.....	70
1.5 Glaucoma	72
1.5.1 Rodent glaucomatous model.....	73
1.5.2 Controversy over the primary site of retinal damage in glaucoma.....	74
1.5.3 Retinal function in glaucoma	75
1.5.4 Piezo channel in glaucoma	76

Chapter 2 Retinal Response to Chemical Injury in the Anterior Segment.....78

2.1 Introduction.....	78
2.2 Methodology	81
2.2.1 Ethics approval	81
2.2.2 Animal preparation	81
2.2.2.1 Mice	81
2.2.2.2 Genotyping.....	82
2.2.2.3 Cornea alkali burn mouse model	83
2.2.3 Intravitreal injection.....	84
2.2.4 Retina preparation.....	85

2.2.5 Patch clamp.....	87
2.2.5.1 Intracellular dye injections.....	87
2.2.5.2 Patch-clamp recording	88
2.2.5.3 Light stimulation.....	89
2.2.6 Immunohistochemistry	91
2.2.6.1 Whole mount for mouse retina	91
2.2.6.2 TUNEL labeling for mouse retina.....	93
2.2.7 Imaging acquisition and processing.....	93
2.3 Results.....	95
2.3.1 Corneal alkali burns result in significant death of retinal ganglion cells.....	95
2.3.2 Gap junction-mediated apoptosis in retina	99
2.3.3 Blocking Cx36-related gap junctions may significantly reduce cell death in the far-peripheral and mid-peripheral retina following six-hour corneal alkali burns: analysis in genetic and pharmacological mice.....	101
2.3.4 No significant morphological changes in ON and OFF α RGCs following six-hour corneal alkali burn	106
2.3.5 Corneal alkali burns reduce light-evoked spike responses in ON and OFF transient α RGCs.....	108
2.3.6 Substantial reduction in light-evoked EPSCs/IPSCs in ON and OFF α RGCs after six-hour corneal alkali burn	110
2.3.7 MFA treatment reduces light sensitivity loss of ON and OFF transient α RGC following a six-hour alkali burn.....	113
2.3.8 The effect of MFA on EPSCs in ON α RGCs.....	116
2.3.9 Microglial activation following corneal alkali burns.....	119
2.4 Discussion	122
2.4.1 Apoptosis of RGCs in corneal alkali burn: A six-hour post-burn model	122
2.4.2 Alkali did not play a key role in the apoptosis of RGCs following corneal alkali burns	123

2.4.3 Microglial activation and RGCs' apoptosis following corneal alkali burns	124
2.4.4 Connexin 36 played a role in acute retinal ganglion cell apoptosis six hours after corneal alkali burn	125
2.4.5 Protecting RGCs from corneal alkali burn with MFA application	126
2.5 Conclusion	128
Chapter 3 Pressure-induced Retinal Ganglion Cell Signal Alterations: Sustained, Acute, and Direct Mechanical Effects.....	129
3.1 Introduction.....	129
3.2 Methodology	132
3.2.1 Animal preparation	132
3.2.1.1 Mouse model of AOH.....	133
3.2.1.2 Mouse model of SOH	135
3.2.2 Measurement in the mouse model	137
3.2.2.1 Intraocular pressure.....	137
3.2.2.2 Electroretinography.....	138
3.2.2.3 Optomotor test	139
3.2.3 Retina preparation.....	140
3.2.4 light stimulation	140
3.2.5 Mechanical pressure on α RGCs.....	140
3.2.6 Patch clamp.....	141
3.2.7 Immunohistochemistry	141
3.3 Results.....	143
3.3.1 IOP elevation of the SOH mouse model.....	143
3.3.2 Decline of inner retinal function in the SOH mouse model.....	145
3.3.3 The loss of α RGCs in the retina of 4-week SOH mice	147
3.3.4 The reduced light sensitivities of four subtypes of α RGCs in mice following 4 weeks of SOH	149

3.3.5 Elevated IOP and decreased contrast sensitivity in the AOH mouse model.....	152
3.3.6 AOH injury results in the loss of the α RGCs.....	154
3.3.7 Unchanged dendritic field and soma size of α RGCs following AOH at 50 minutes, 2 weeks, and 4 weeks post-elevation	157
3.3.8 After four weeks AOH, there was a significant decrease in the maximum light response of α RGCs	159
3.3.9 Increase in α RGC spikes under direct mechanical pressure applied by pressure controller	163
3.3.10 α RGC amplitude reduction without reversal potential changes under pressure: three-group analysis of WT mice before and after micropipette compression, and 4-week SOH mice.....	166
3.4 Discussion	171
3.4.1 Retinal ganglion cell loss in two ocular hypertension mouse models	171
3.4.2 Compromised retinal visual function in AOH and SOH mice model.....	172
3.4.3 Mechanical pressure on α RGC	173
3.5 Conclusion	175
Chapter 4 Overall Conclusion.....	176
Reference	178

List of Figures

Figure 1.1 The structure of the retina.....	21
Figure 1.2 The main cell classes in the mouse retina.....	22
Figure 1.3 Phototransduction cascade in carp rods and cones.....	26
Figure 1.4 The three rod pathways in the mammalian retina	28
Figure 1.5 The cone pathway	29
Figure 1.6 Illustration of the organization of the bipolar cells in the mouse retina	32
Figure 1.7 Illustration of narrow-and medium-field amacrine cells	36
Figure 1.8 Exemplary morphologies of RGCs.	43
Figure 1.9 A summary of morphological, physiological, and molecular features for the four types of α RGCs	44
Figure 1.10 Illustration of Gap junctions (a) and connexin subunits (b).....	51
Figure 1.11 Schematic of the electrical synaptic circuitry of the retina	56
Figure 1.12 Connexin36 in the three rod pathways	59
Figure 1.13 Schematic representation of apoptosis	67
Figure 2.2.2.3 Illustration of mouse cornea following alkali-induced injury	84
Figure 2.3.1 The apoptosis of retinal ganglion cells resulting from corneal alkali burns.....	99
Figure 2.3.2 Gap junction-mediated apoptosis in adjacent neuronal cells following cytochrome C injection.....	100
Figure 2.3.3 Retinal damage following six-hour corneal alkali burns.....	105
Figure 2.3.4 ON and OFF α RGCs in the retinas of WT mice before and after six-hour corneal alkali burns.....	107
Figure 2.3.5 Light responses of α RGCs in WT and six-hour Alkali Burn Groups.....	110
Figure 2.3.6 Light-evoked EPSCs and IPSCs in ON and OFF α RGCs of WT and six-hour alkali burn groups.	113
Figure 2.3.7 Light responses of ON and OFF transient α RGCs in six-hour alkali burn of Ringer and MFA-treated groups.	115
Figure 2.3.8 Light-evoked EPSCs and IPSCs in ON and OFF α RGCs of Wild-type control and six-hour alkali burn groups.	119

Figure 2.3.9 Microglial changes following a six-hour corneal alkali burn.....	121
Figure 3.2.1.1 Illustration of a sharp micropipette being inserted into the anterior chamber.	134
Figure 3.2.1.2 Illustration of a homemade micropipette.....	136
Figure 3.3.1 IOP elevation of the SOH mouse model	144
Figure 3.3.2 ERG responses of control and 4-week SOH mice.....	146
Figure 3.3.3 The number of RGCs, AII ACs of control and 4 weeks of SOH mice.....	149
Figure 3.3.4 The effect of 4-week SOH on the light intensity of four subtypes of α RGCs.....	152
Figure 3.3.5 IOP measurement and optomotor response after AOH injury.	154
Figure 3.3.6 The quantitative analysis of α RGCs in the retinas of WT mice following AOH at 50 minutes, 2 weeks, and 4 weeks post-elevation.	156
Figure 3.3.7 The morphology of ON and OFF α RGCs in the retinas of WT mice following AOH at 50 minutes, 2 weeks, and 4 weeks post-elevation.....	159
Figure 3.3.8 Light response after AOH.....	163
Figure 3.3.9 Mechanical pressure on α RGCs.	166
Figure 3.3.10 Light-evoked currents of α RGCs under different holding potentials in control, acute mechanical pressure, and 4-week SOH groups.	170

List of Tables

Table 1.1 Roper-Hall classification for ocular chemical injury	63
Table 1.2 Dua classification for ocular chemical injury	63
Table 2.1 Antibodies used in the studies	92
Table 2.2 The statistical significance symbols used in the analysis.....	94

List of Abbreviations

Abbreviation	Explanation
AC	Amacrine cell
BC	Bipolar Cell
CCW	Counterclockwise
CNS	Central Nervous System
cpd	Cycle Per degree
Cx	Connexin
CSARF	Center-Surround Antagonistic Receptive Fields
DACs	Dopaminergic Amacrine cells
DBC	Depolarized Bipolar Cells
EPSCs	Excitatory Postsynaptic Currents
ffERG	Full-field Electroretinogram
GCL	Ganglion Cell Layer
HBC	Hyperpolarized Bipolar Cell
HC	Horizontal Cell
INL	Inner Nuclear Layer
IOP	Intraocular Pressure
IPL	Inner Plexiform Layer
IPSCs	Inhibitory Postsynaptic Currents
MFA	Meclofenamic Acid
nSTR	Negative Scotopic Threshold Response
OFFs- α RGC	OFF Sustained Alpha Retinal Ganglion Cell
OFFt- α RGC	OFF Transient Alpha Retinal GGanglion Cell
ONH	Optic Nerve Head
ONL	Outer Nuclear Layer
ONs- α RGC	ON Sustained Alpha Retinal Ganglion Cell
ONt- α RGC	ON Transient Alpha Retinal Ganglion Cell
OPL	Outer Plexiform Layer
PDE	Phosphodiesterase
PSCs	Postsynaptic Currents

PSTH	Peristimulus Histogram
pSTR	Positive Scotopic Threshold Response
RGC	Retinal Ganglion Cell
SEM	Standard Error of the Mean
TH	Tyrosine hydroxylase
TNF- α	Tumor Necrosis Factor Alpha
TUNEL	Tdt-mediated dUTP-X-nick end labeling
WT	Wild type
α RGC	Alpha Retinal Ganglion Cell
λ	Wavelength

Chapter1 Introduction

1.1 The anatomy of the eye

Eyes are organs of the visual system, including the spheroidal eyeball containing sense receptors for vision, protective accessory visual structures, and visual pathways integrated with the cerebral cortex. The eyeball is typically divided into anterior and posterior segments in mammals. The former consists of the cornea, iris, anterior chamber, and lens, while the latter constitutes the vitreous body, retina, choroid, and sclera. Due to different embryonic origins, the cornea from the surface ectoderm and the retina from the anterior neural plate, and different developmental signals are mediated, the anterior segment and posterior segment are not synchronous in the process of development.

1.1.1 Cornea

The human cornea holds five layers: cornea epithelium, Bowman's layer, corneal stroma, Descemet's membrane, and corneal endothelium.

The mouse cornea has five layers as well: epithelium, anterior limiting lamina, stroma, posterior limiting lamina, and endothelium.

1.1.2 Retina

The retina is the starting point of human vision formation and an indispensable part of the visual system. Any damage to the retina affects the transmission of light signals, thereby altering human perception of the external environment. As a common site for ocular diseases, the retina is susceptible to various pathologies, including macular degeneration, glaucoma, diabetic retinopathy, and retinal detachment. These retinal diseases not only severely impact patients' quality of life but also place an enormous burden on healthcare systems.

1.2 Retina structure and neurons

The retina consists of ten distinct layers organized from outer to inner, with five principal neuronal cell types (photoreceptors, horizontal cells, bipolar cells, amacrine cells, and ganglion cells) and their synaptic connections interspersed throughout, as shown in **Fig. 1.1** (Yang et al., 2021). This sophisticated architecture establishes the foundation for the precise capture and transmission of visual information.

The neurons in the retina involved in processing visual information are arranged like a sandwich: photoreceptors capture the initial light signals and pass the information to bipolar cells, which are ultimately collected by retinal ganglion cells (RGCs) and transmitted to the brain, as shown in **Fig. 1.2** (Sanes & Masland, 2015).

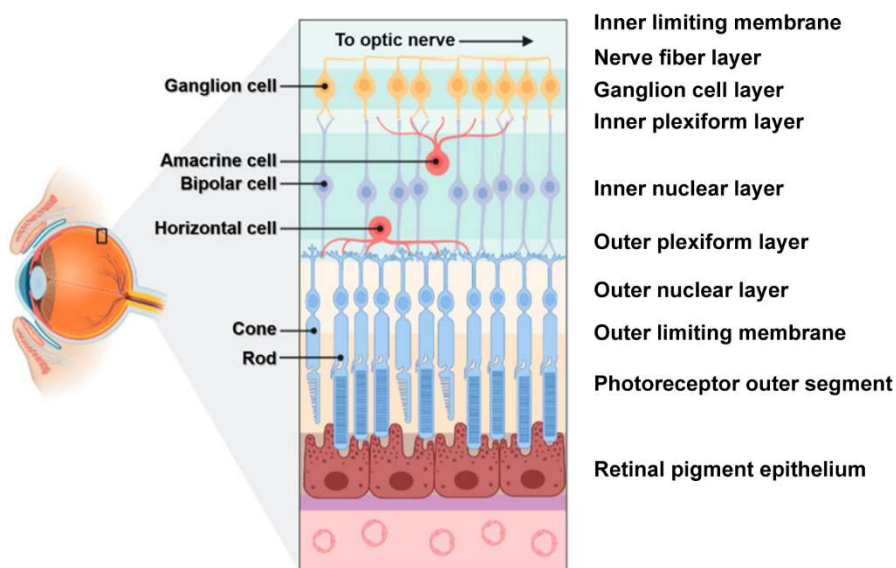


Figure 1.1 The structure of the retina, modified from (Yang et al., 2021). The mammalian retina exhibits a highly organized laminar structure comprising 10 distinct layers that house five principal neuronal cell types responsible for the initial stages of visual information processing. These layers include the inner limiting membrane, nerve fiber layer, ganglion cell layer, inner plexiform layer,

inner nuclear layer, outer plexiform layer, outer nuclear layer, outer limiting membrane, photoreceptor layer, and retinal pigment epithelium. Five neurons include the photoreceptor (cone and rod), horizontal cell, bipolar cell, amacrine cell, and ganglion cell.

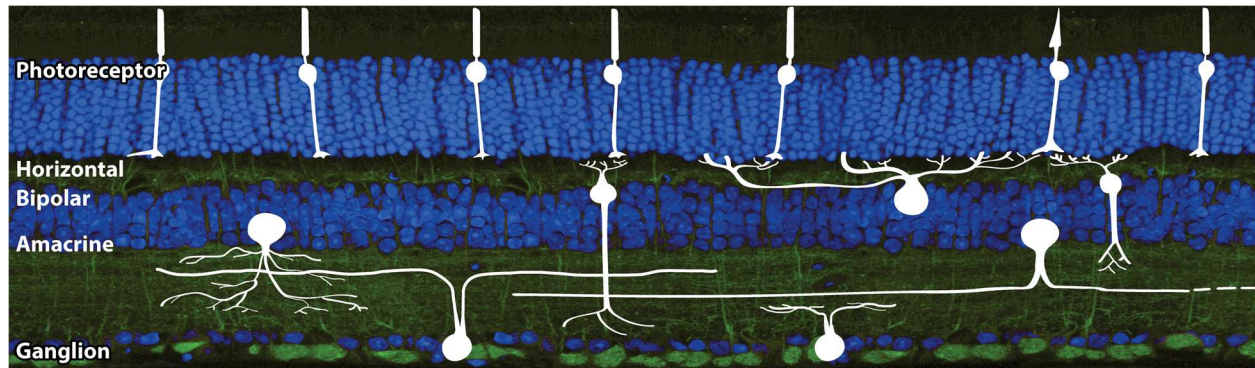


Figure 1.2 The main cell classes in the mouse retina, modified from (Sanes & Masland, 2015). The retina has a sandwich-like structure: photoreceptors capture the initial light signals and pass the information to bipolar cells. Horizontal cells and amacrine cells provide lateral inhibition and signal modulation. While retinal ganglion cells ultimately collect all the processed information and transmit the decoded signals to the central nervous system.

1.2.1 Photoreceptors

1.2.1.1 Rod and cone cells

All mammals have photoreceptors in their retinas. Despite being located in the outer layer of the retina, photoreceptors are actually the first structures to detect light and are responsible for converting light signals into electrochemical signals.

Photoreceptors are categorized into two types: rods and cones. When viewed in vertical retinal sections, these two cell types can be clearly distinguished from one another based on their distinctive morphological characteristics. This is because, although both cell types contain four structural components (outer segment, inner segment, nucleus, and synaptic terminal), the morphology of their outer segments differs significantly. Rod outer segments are cylindrical in shape with numerous discrete membranous discs, whereas cone outer segments exhibit a distinctive conical structure. The differences between rods and cones are reflected in their responses to light stimuli as well. Rods, with their extraordinary sensitivity to light and ability to respond to a single photon, are highly specialized for scotopic vision under low-light conditions. Cones are responsible for photopic vision with relatively lower light sensitivity but faster light response. Moreover, rods exhibit a delay in response after the cessation of light, whereas cones do not. For this reason, our eyes are better able to detect moving objects during daylight, while it becomes much more difficult under low-light conditions (Kawamura & Tachibanaki, 2008). Gap junctions were observed in mammalian photoreceptors, contributing to an improved signal-to-noise ratio.

In general, rods outnumber cones. Mice are typical rod-dominated animals, accounting for approximately 97% of rods in the retina (Jeon et al., 1998). The substantial numerical difference

has positioned cones in a relatively less understood role compared to rods. For humans, cones concentrate in the fovea and provide the high visual acuity necessary for detailed central vision. In contrast, mice lack a fovea structure, so their cones are not concentrated in any specific region but are distributed differently across the retina. In mice, there is only one type of rod photoreceptor, while cones exist in two kinds. The former contains rhodopsin, while the latter contains cone opsins: short wavelength-sensitive opsins (S-opsins) and middle wavelength-sensitive opsins (M-opsins). Mouse is unusual in that the majority of cones co-express both S- and M-opsins in the same cone (Applebury et al., 2000; Lyubarsky et al., 1999), while the remaining cones exclusively express M-opsins in the far dorsal retina, which is, however, overall expressed at lower levels compared to S-opsins (Applebury et al., 2000). Due to mice lacking long-wavelength cones, laboratories commonly utilize red light to maintain dark adaptation during experiments. However, when the light intensity becomes sufficiently bright, mice will still exhibit responses to this illumination (Peirson et al., 2018). The peak sensitivity of rhodopsin is near 500 nm, while the peak sensitivities of S- and M-cone pigments are at 360 nm and 508 nm in mice, respectively (Jacobs et al., 1991; Lyubarsky et al., 1999; Sun et al., 1997).

The details of phototransduction in rods are well-established, with a similar process occurring in cones (Fu & Yau, 2007). Rod photoreceptors convert photons into electrochemical signals through a process called phototransduction, which is the first step in visual information processing, as shown in **Fig. 1.3** (Kawamura & Tachibanaki, 2008). When photons are absorbed by rhodopsin, they trigger the isomerization of the chromophore 11-cis-retinal to all-trans-retinal. This photoexcited rhodopsin interacts with the G protein transducin, which in turn activates phosphodiesterase (PDE). PDE then hydrolyzes cyclic GMP (cGMP) to GMP, resulting in decreased cytoplasmic cGMP concentration. Since cGMP keeps the opening of cation channels,

the reduction in cGMP levels causes these channels to close, thereby reducing the influx of Na^+ and Ca^{2+} ions. This cascade leads to hyperpolarization of the photoreceptor cell. The resulting voltage signal spreads to the synaptic terminal, modulating glutamate release and ultimately transmitting visual information to downstream retinal neurons, including the bipolar and horizontal cells.

In darkness, photoreceptor cells, including rods and cones, exhibit a steady inward current, commonly referred to as the "dark current," which is mediated by a cation conductance located on the outer segment membrane. This current depolarizes the membrane potential and facilitates the continuous release of glutamate at the synapse. Upon exposure to light, the cation conductance, which is composed of cGMP-gated channels termed the "light-sensitive" conductance, closes, resulting in the cessation of the dark current. This results in a hyperpolarization of the membrane, which subsequently reduces or terminates the release of glutamate. The resulting changes in glutamate release are then processed by downstream neurons in the retina before being transmitted to higher visual centers in the brain for further interpretation and integration (Fu & Yau, 2007).

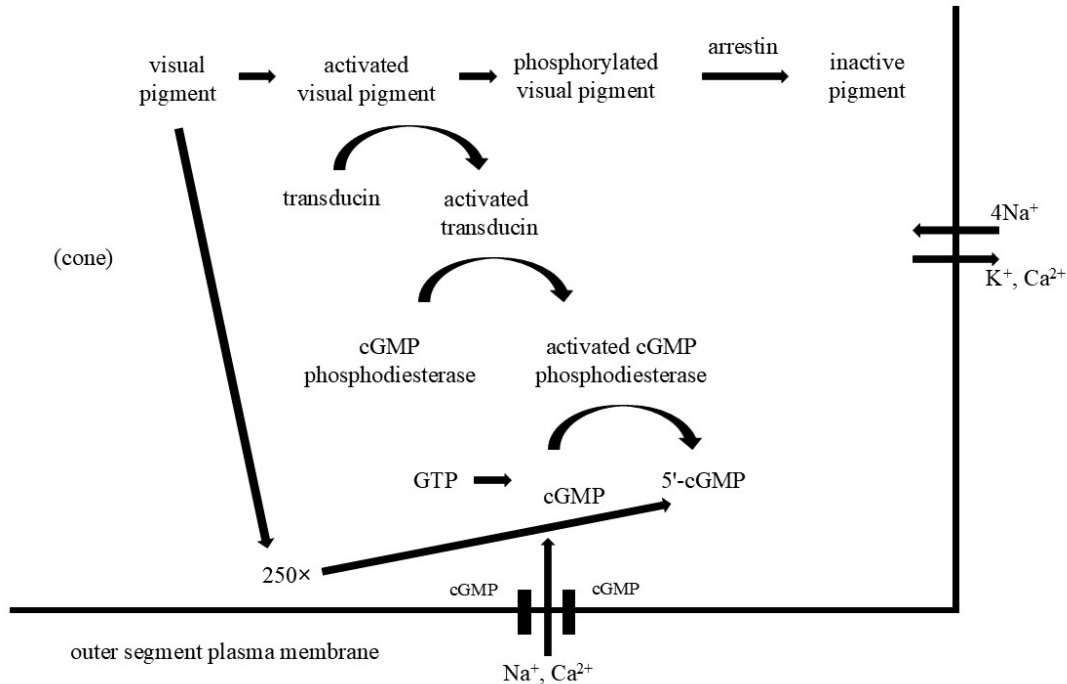


Figure 1.3 Phototransduction cascade in carp rods and cones, modified from (Kawamura & Tachibanaki, 2008). Photoreceptor outer segments utilize a cGMP hydrolysis cascade to initiate cellular responses to light stimulation. See details in the text.

1.2.1.2 Rod and cone pathway

There are three rod pathways proven in the mouse retina: the primary rod pathway, the alternative rod pathway, and the third rod pathway (Grimes et al., 2018; Pan et al., 2016), as shown in **Fig. 1.4** (Pan et al., 2016).

In the primary rod pathway, rods synapse with a class of rod bipolar cells, and the latter sequentially synapse with AII amacrine cells. Whereafter, AII amacrine cells transmit the ON streams to the

ON cone bipolar cells via excitatory gap junctions, meanwhile transmitting the OFF signals to the OFF cone bipolar cells by inhibitory glycinergic synapses, finally reaching ON RGCs and OFF RGCs, respectively.

In an alternative rod pathway, the signal can directly pass from rods to cones via gap junctions. This rod excitation can convey to the ON cone bipolar cells and the OFF cone bipolar cells, followed by the corresponding ON RGCs and OFF RGCs.

In the third rod pathway, a rare and unique pathway is formed between the rod and a specialized OFF cone bipolar cell. It is speculated that the rod also synapses with a type of ON cone bipolar cell.

The cone pathways in mammalian retina demonstrate greater directness and spatial specificity compared to rod pathways in **Fig. 1.4** (Kolb, 1995). Unlike rod circuits that require intermediate amacrine cell processing, cone bipolar cells synapse directly onto ganglion cell dendrites. ON cone bipolar cells connect with ON RGCs, while OFF cone bipolar cells establish synapses with OFF RGCs. This architectural difference results in more narrow-field and convergent cone pathways (Kolb, 1995).

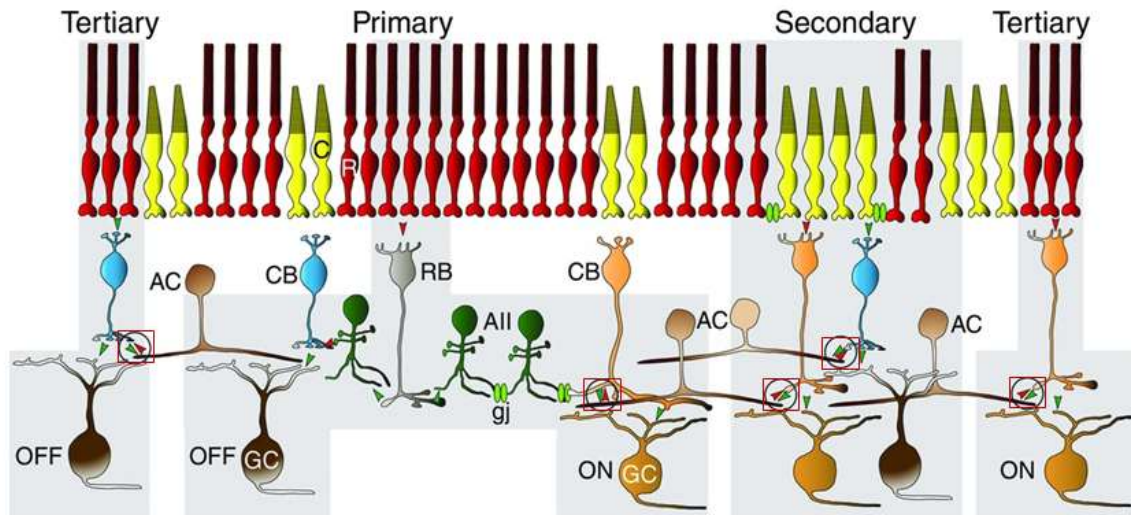


Figure 1.4 The three rod pathways in the mammalian retina, modified from (Pan et al., 2016).

The primary rod pathway follows a circuit: rods $\rightarrow$ RB $\rightarrow$ AII $\rightarrow$ CB $\rightarrow$ GC. The secondary pathway involves: rods $\rightarrow$ cones $\rightarrow$ CB $\rightarrow$ GC. The tertiary pathway represents a more direct route: rods $\rightarrow$ CB $\rightarrow$ GC. AC, amacrine cell; AII, AII amacrine cell; CB, cone bipolar cell; RB, rod bipolar cell; ON RGC, ON retinal ganglion cell; OFF RGC, OFF retinal ganglion cell; gj, gap junction.

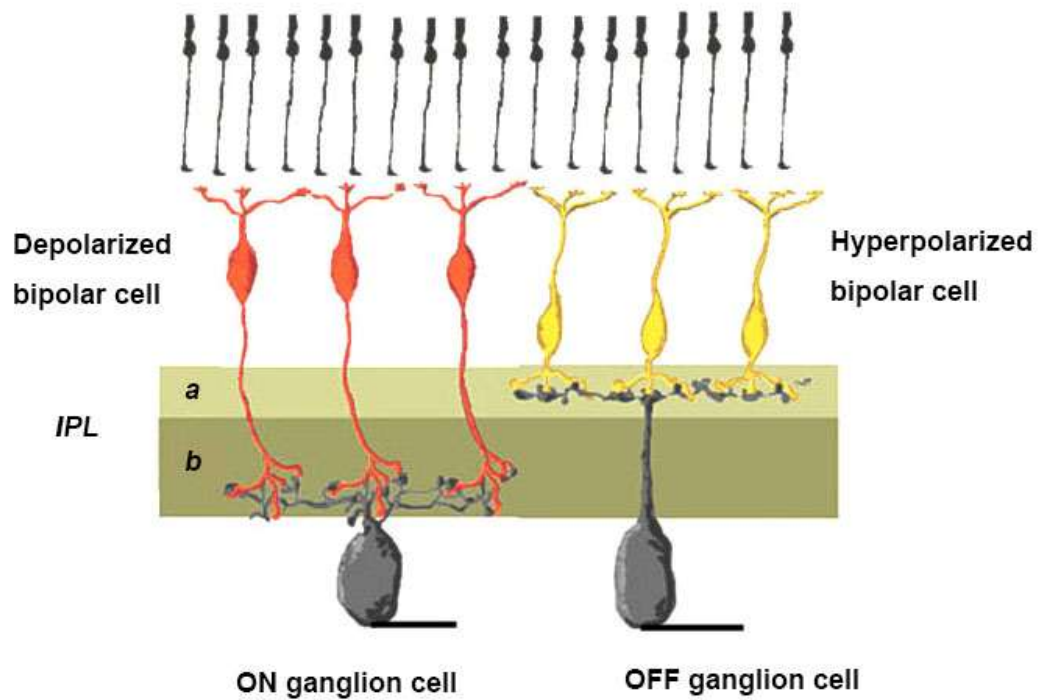


Figure 1.5 The cone pathway, modified from (Kolb, 1995). Cones form synapses with both depolarized bipolar cells and hyperpolarized bipolar cells, which subsequently connect to ON and OFF retinal ganglion cells, respectively.

1.2.2 Bipolar cell

The morphology of retinal bipolar cells (BCs) features two protrusions extending from the neuron in opposite directions, with one upward and one downward when viewed in cross-section. In the vertebrate retina, the soma of the BC is situated in the inner nuclear layer (INL), with its two protrusions going up to reach photoreceptors and down to reach the inner plexiform layer (IPL), thus relaying signals from the photoreceptors to the IPL, where it provides input to RGCs and ACs. All major BCs in the mouse retina seem to have been discovered: there are 12 types of cone inputting BCs, and only 1 type of rod-driven BC, which is mainly differentiated based on their IPL stratification level (Euler et al., 2014; Helmstaedter et al., 2013), as shown in **Fig. 1.6** (Euler et al., 2014). Each cone pedicle establishes synaptic connections with a minimum of 10 distinct BCs (Wässle et al., 2009). These distinct types of BCs contribute to transforming the photoreceptor signals into different channels, which encode specific stimulus properties, such as response polarity (ON and OFF), speed (transient and sustained responses), chromatic sensitivity, and spatial receptive field (Breuninger et al., 2011; Chalupa & Günhan, 2004; DeVries et al., 2006; Mataruga et al., 2007; Roska & Werblin, 2001). Therefore, BCs are considered the first step in diverging visual information and visual pathways.

In light, the glutamates released from photoreceptors are reduced, which excites the depolarizing BC and inhibits the hyperpolarizing BC. We typically refer to these two types of cells as ON and OFF BCs as well. In general, ON BCs outnumber OFF BCs in most mammals. This polarity of their light response is primarily caused by the different glutamate receptors present on these two types of BCs. ON BCs form invaginating contact with cone pedicles by inhibitory metabotropic glutamate receptors (mGluR), especially mGluR6. Glutamate closes cation channels in ON BCs via the mGluR6 receptor cascade, which induces the closure of transient receptor potential cation

channel subfamily M member 1 (TRPM1) (Koike et al., 2010). Finally, it implements the conversion of hyperpolarization signals transmitted from photoreceptors into depolarization in BCs. By contrast, OFF BCs form flat contact with the cone pedicle base via excitatory ionotropic glutamate receptors (iGluR). Glutamate opens cation channels in OFF BCs via AMPA/KA receptors (DeVries, 2000; DeVries et al., 2006). When the light is on, reduced glutamate closes cation channels, leading to membrane hyperpolarization of OFF BCs. This is the only location where membrane polarity is reversed, with downstream cells following this polarity further.

OFF BCs stratify their axonal terminals in the outer sublamina of IPL, while ON BCs predominantly stratify in the inner sublamina (Wu, 2010). In the mammalian retina, the IPL is subdivided into five distinct strata of approximately equal thickness, with the outer strata corresponding to the OFF sublamina (strata 1 and 2) and the inner strata to the ON sublamina (strata 3-5) (Famiglietti & Kolb, 1976). BC types that exhibit more transient light responses tend to stratify near the middle of the IPL, whereas those with more sustained responses are typically located closer to the IPL borders (Baden et al., 2013; Borghuis et al., 2013). This distinct stratification pattern reflects the functional organization of the retina, in which the ON and OFF pathways are anatomically and functionally segregated to process visual signals.

BCs exhibit center-surround antagonistic receptive fields (CSARF), a key feature that allows them to encode spatial information effectively. In this arrangement, the receptive field is divided into a central region (center) and a surrounding region (surround), with the two regions responding oppositely to light stimuli. The central part of the receptive field directly responds to light, while the surrounding region exhibits an opposite response to the center, corresponding to ON-center/OFF-surround (depolarized) BCs and OFF-center/ON-surround (hyperpolarized) BCs,

respectively. The surround's inhibitory effect is achieved through lateral inhibition mediated by horizontal cells and ACs, which sharpens contrast, detects edges, and filters out noise.

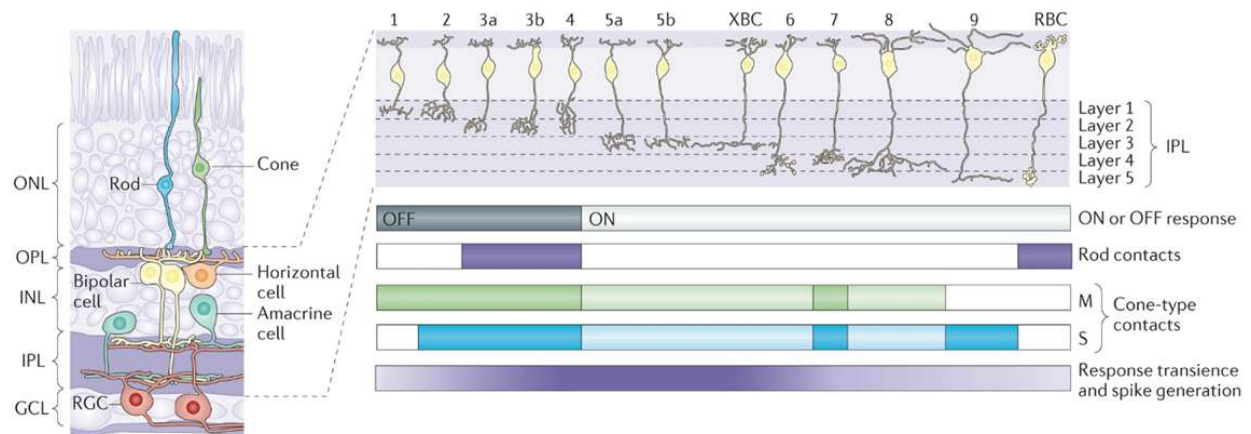


Figure 1.6 Illustration of the organization of the bipolar cells in the mouse retina, modified from (Euler et al., 2014). Thirteen bipolar cell types have been identified, with their dendrites distributed in different layers of the IPL and exhibiting different characteristics, including: ON or OFF responses, rod contacts, cone-type contacts, response transience, and spike generation.

1.2.3 Horizontal cell

Retinal horizontal cells (HCs) occupy positions within the inner nuclear layer (INL). In the human retina, there are three types of HCs being observed: HI type, HII type, and HIII type (Kolb et al., 1994). Zebrafish retinal HCs comprise three distinct types that can be unequivocally identified: H1/2 and H3 cells, which connect to cones, and a third type that connects to rods (Connaughton et al., 2004). The rabbit retinal HC system consists of A-HCs and B-HCs (Hack & Peichl, 1999). Only one kind of B-type (axon-bearing type) of HC has been found in some rodent retina, which is in the INL (Masland, 2001; Peichl & González-Soriano, 1994; Peichl et al., 1998).

In addition to having a soma and dendritic structure, a distinctive structural feature of mouse retinal HC is the axonal terminal. In axon-bearing HCs, the axons do not transmit neural signals but instead provide metabolic support to a functionally independent "axon terminal system." In mice, this system is involved explicitly in mediating lateral inhibition among rod photoreceptors. Meanwhile, the dendritic arbor of the HC serves the same role exclusively for cone photoreceptors. Therefore, HCs modulate information from photoreceptors, rather than transmitting the signal.

HCs are strongly homocellularely coupled together through connexin57 (Cx57) homotypic gap junctions, forming an extensive network with large receptive fields to integrate visual signals (Kaneko, 1971; Shelley et al., 2006). Electrical synapses are likely involved in the transmission of feedforward antagonistic surround signals from horizontal cells to depolarizing bipolar cells (Zhang et al., 2006).

HCs are the basis for the formation of center-surround antagonistic receptive fields. When light suppresses glutamate release from photoreceptors, AMPA receptors on HCs are modulated, allowing the HCs to hyperpolarize. Three possible mechanisms suggest that HCs provide sign-inverting feedback to cones: gamma-aminobutyric acid (GABA) release, ephaptic modulation, and

cone calcium currents modulation (Thoreson & Mangel, 2012). Additionally, feedback and feedforward pathways exist between HCs and BCs. Experimental evidence demonstrates that depletion of HCs disrupts the formation of antagonistic center-surround receptive fields in RGCs and diminishes the diversity of both ON and OFF responses in RGCs (Chaya et al., 2017). These feedback and feedforward mechanisms from the HCs indicate that HCs play essential roles in the retinal circuits and visual processing.

1.2.4 Amacrine cell

Cajal first discovered and named the amacrine cell (AC) in the avian retina. After this, these neurons with unique morphology have been extensively studied and confirmed in multiple species. ACs are positioned in the inner nuclear layer as a kind of interneuron receiving visual signals from BCs and neighboring ACs, thus transferring the processed information to the retinal ganglion cells. More than 60 types of distinct ACs have been discovered in the mouse retina, which could be classified using multiple criteria. According to the dendritic field size, ACs are divided into narrow field (30-150 μm), medium field (150-500 μm), and wide field (>500 μm) (Kolb et al., 1981; Lin & Masland, 2006; Masland, 2012b; Pang et al., 2012), as shown in **Fig. 1.7** (Masland, 2012b). Based on dendritic stratification patterns in IPL, they are categorized as monostратified, bistratified, multistratified, etc. (Lee et al., 2015).

Neurochemically, ACs utilize a diverse range of neurotransmitters to regulate retinal circuitry, including glycine, GABA, dopamine, acetylcholine, and nitric oxide. The majority of retinal ACs are divided into two categories: GABAergic ACs with wide dendritic fields, and glycinergic ACs with narrow dendritic fields (de Vries et al., 2011; Menger et al., 1998). Among these, GABAergic cells not only express GABA but also generally emit one or more other types of transmitters, including dopamine, acetylcholine, nitric oxide, and others. In addition to neurotransmitters, many ACs communicate with RGCs and other ACs via gap junctions, and the transmission speed of gap junctions is considered faster than that of neurotransmitters (Connors & Long, 2004).

ACs are one of the primary neuron types in the retina, receiving excitatory signals from BCs and inhibitory signals from other ACs (Marc & Liu, 2000). Meanwhile, ACs deliver inhibitory synaptic input to the terminals of BCs, the dendrites of RGCs, and other ACs (Diamond, 2017). In the vertebrate retina, ACs are classically regarded as having an inhibitory role in modulating the

receptive field surrounding, which enables shaping both spatial and temporal features (Diamond, 2017). Currently, two major pathways have been studied: on one hand, feedback inhibition on BCs, and on the other hand, feedforward inhibition on RGCs (Flores-Herr et al., 2001; Lee et al., 2016; Tachibana & Kaneko, 1988). This inhibitory function is primarily due to the neurotransmitter expression patterns in ACs (Eggers & Lukasiewicz, 2006). Notably, inhibiting one of the subtypes of inhibitory receptors can lead to an overall increase in inhibitory signaling. Glycine receptor blockade results in the enhancement of GABAergic signaling in both feedforward and feedback pathways (Zhang et al., 1997), indicating that disinhibition occurs in ACs. Therefore, ACs are involved in the visual pathway, greatly contributing to deciphering retinal computation.

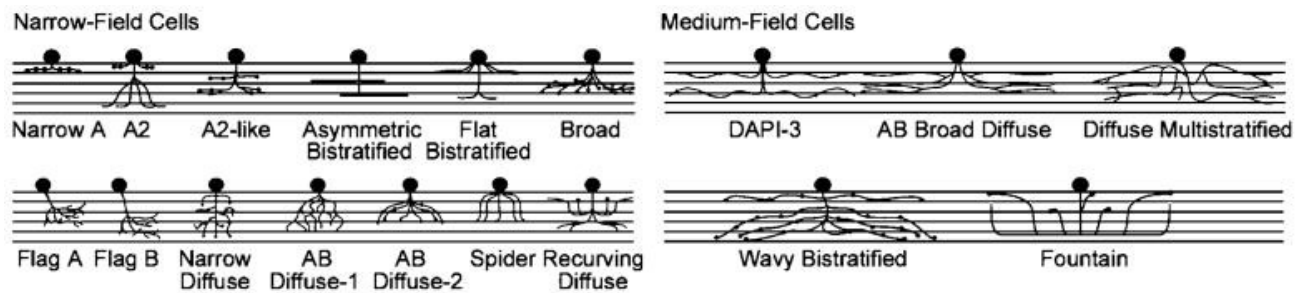


Figure 1.7 Illustration of narrow- and medium-field amacrine cells (Masland, 2012b). See details in the text.

1.2.4.1 AII amacrine cell

AII amacrine cells (AII ACs) are highly characteristic glycinergic amacrine cells with narrow fields in the mammalian retina, functioning in both photopic and scotopic conditions (Diamond, 2017; Kolb & Famiglietti, 1974; Pourcho & Goebel, 1985). Among glycinergic ACs, the AII

subtype is predominant, representing approximately one-fifth to one-third of this neuronal population in the rat retina (Menger et al., 1998). AII ACs are located in the inner nuclear layer, which could be labeled by Prox1 and Disabled1 immunostaining (Lee et al., 2006; Rice & Curran, 2000). Prox1-labeled amacrine cells were found in the whole area of the retina, including the inferior, superior, temporal, and nasal regions. There are 3887 ± 160 cells/mm² AII ACs immunostained in the region adjacent to the optic nerve, which represents the area of highest density (Pérez de Sevilla Müller et al., 2017).

AII ACs play an essential role in the primary rod pathway, receiving signals from rod BCs and subsequently transmitting this information to the cone pathway. As a result, it is also called a rod-AC. AII ACs serve as critical intermediaries in the visual system, establishing essential neural bridges between rod and cone signaling pathways. Additionally, they segregate the visual signals into ON and OFF components through distinct synaptic mechanisms in scotopic conditions: forming electrical synapses (gap junctions) with ON cone BCs while communicating with OFF BCs and OFF RGCs via inhibitory glycinergic synapses (Dacheux & Raviola, 1986; Famiglietti & Kolb, 1975; Kolb & Famiglietti, 1974; Manookin et al., 2008; Murphy & Rieke, 2008). This character is likely to be strongly linked to the morphology of AII ACs, presenting arboreal dendrites on both the ON sublamina and the OFF sublamina of the IPL (Famiglietti & Kolb, 1975). Notably, ON inhibitory signals would be relayed from AII ACs to ganglion cells through gap junction-mediated pathways in photopic condition (Münch et al., 2009).

The light-sensing capabilities of AII ACs exhibit exceptional sensitivity. Furthermore, AII ACs establish homotypic coupling with neighboring AII ACs via gap junctions containing connexin 36 (Cx36), creating a functional network that enhances signal processing. Interestingly, AII ACs exhibit minimal coupling under conditions of complete darkness, but their coupling strength

increases significantly within scotopic light levels (Bloomfield & Völgyi, 2004). When illumination reaches photopic levels, AII ACs undergo decoupling once more through the dephosphorylation of Cx36 by phosphatases that are activated via dopamine receptors (Kothmann et al., 2009).

1.2.4.2 Dopaminergic amacrine cell

Dopaminergic amacrine cells (DACs) are located in the innermost part of the INL, whose dendrites and axons are distributed within sublamina 1 of the IPL (Ballesta et al., 1984; Keeley & Reese, 2010; Wang et al., 1990). Dopaminergic cells represent one of the most sparsely distributed cell populations in the retina, comprising fewer than 0.01% of the total retinal neuronal population, which were labeled by tyrosine hydroxylase (TH) (Zhang et al., 2004; Zhang et al., 2024).

DACs in the retina release dopamine according to a circadian pattern, with elevated concentrations during daytime and reduced levels throughout the night (Doyle et al., 2002). Dopamine in the retina functions both as a circadian clock resetting mechanism and as a key modulator that governs high-acuity vision, light-adapted visual processing, and photoreceptor cell coupling (Besharse & McMahon, 2016; Jackson et al., 2012; Jin et al., 2015; Ribelayga et al., 2008). Through its interaction with five distinct G-protein-coupled dopamine receptors, dopamine modulates voltage-gated ion channels and modifies both chemical and electrical forms of synaptic transmission. These five dopamine G-protein-coupled receptors segregate into two functional subfamilies distinguished by their opposite effects on adenylyl cyclase - the stimulatory D1-like subfamily (D1, D5) and the inhibitory D2-like subfamily (D2, D3, D4) (Jackson et al., 2012). D1 dopamine receptors are present on horizontal cells and subsets of BCs, ACs, and RGCs, while the D2 receptor

subfamily shows D2 expression on DACs and D4 expression predominantly on photoreceptors (Cohen et al., 1992; Derouiche & Asan, 1999; Farshi et al., 2016; Flood & Eggers, 2021; Klitten et al., 2008; Veruki & Wässle, 1996).

Light responses in DACs occur via both the rod and cone pathways, with the rod pathway playing a documented role in dopamine regulation (Zhang et al., 2008; Zhao et al., 2017). DACs display a diverse range of firing patterns: two forms of intrinsic bursting during darkness that are influenced by inhibitory synapses, two types of light responses (transient and sustained ON responses), and activity that occurs independently of light stimulation, with each pattern tailored for specific visual dopaminergic roles (Zhang et al., 2007). Transient dopamine neurons receive their light-evoked responses via ON BCs circuits that are activated by either rod or cone photoreceptors (Zhang et al., 2007). The melanopsin-mediated phototransduction pathway in intrinsically photosensitive RGCs appears to generate sustained light responses in dopaminergic neurons, which constitute the primary neuromodulatory network of the vertebrate retina (Barnard et al., 2006; Brainard & Morgan, 1987; Godley & Wurtman, 1988; Zhang et al., 2008).

1.2.5 Retinal ganglion cell

The retinal ganglion cell (RGC) collects visual signals from the upstream pathway and parallel channels through its abundant dendrites, thus delivering this coded vision information to the visual cortex in the brain by forming an axon. RGCs, which are critical as the final step in transmitting visual information from the retina, can lead to vision loss, reduced visual function, or even blindness when damaged.

1.2.5.1 Classification of retinal ganglion cells

Structurally, a retinal ganglion cell consists of three parts: the soma, which is located in the ganglion cell layer (GCL); the dendritic structure, which branches and forms flattened arbors in different layers of the IPL to connect with upstream neurons; and the axon, which forms the optic nerve, serving as the only pathway through which the brain receives external visual information. Different types of retinal ganglion cells respond to distinct visual features, providing the brain's visual system with a set of parallel, highly processed visual information, which highlights the significance of ganglion cell classification studies.

Through extensive investigation using dye injections, genetic techniques, immunofluorescence labeling, and light response analysis, mammalian RGCs have been roughly classified based on morphology, gene expression, spacing, and physiological properties. Neuronal tracers with cell injections to distinguish 22 subtypes of RGCs in the wild-type mice based on the somatic and dendritic morphology of different subtypes (Völgyi et al., 2009). Retinal ganglion cells are classified into 15 types based on genetic definitions and dendritic length distributions, revealing

the correspondence between genetic and structural cell types. Approximately 40 distinct RGC types could be categorized based on their functional response features, as shown in **Fig. 1.8** (Baden et al., 2016). There are around 40 different subtypes of RGCs classified by the single-cell transcriptome profiling in the mouse retina (Rheaume et al., 2018).

In the mammalian retina, RGC functional diversity emerges from multiple regulations, with the main contribution coming from the parallel processing of photoreceptor signals by distinct bipolar cell subtypes (Masland, 2012a; Wässle, 2004). Furthermore, diversity is shaped by the specific dendritic and receptor characteristics of individual RGCs (Bae et al., 2018; Brandstätter et al., 1998; Poleg-Polsky & Diamond, 2011; Schachter et al., 2010), as well as by inhibitory modulation from amacrine cell networks (Olstedal et al., 2007). Although multielectrode arrays can capture electrical activity from many RGCs concurrently, they provide limited research value because researchers cannot identify the molecular composition or structural characteristics of the individual cells being recorded. Consequently, patch clamp techniques have gained broader application, while two-photon calcium imaging has similarly garnered significant research interest (Baden et al., 2013).

Previous studies have shown that some RGCs can not only detect light-on and light-off signals but also recognize different attributes of images, including spatial contrast, motion, and textures (Kim et al., 2021; Sanes & Masland, 2015). Four types of ON-OFF directionally selective ganglion cells were described in mice and rabbits, characterized by their special responses to stimuli of a selective direction, including ON and OFF responses (Barlow et al., 1964; Barlow & Levick, 1965). Directional selectivity manifests even when stimuli are considerably smaller than the entire receptive field, indicating that directional reporting occurs regardless of stimulus position within the receptive field (Barlow & Levick, 1965). While mechanistic details continue to emerge, the core machinery underlying this directional discrimination appears to involve selective synaptic

input from the directionally tuned processes of starburst amacrine cells (Masland, 2012a; Vaney et al., 2012). Different features were described in three types of ON Directionally Selective Ganglion Cells (ON DSGCs), which only respond at the ON set. In addition, lacking OFF responses causes these cells to be responsive to much slower-moving stimuli than ON-OFF cells. The synaptic organization reveals another key distinction: these cells restrict their single dendritic layer to interactions with ON starburst cells in the IPL, whereas ON-OFF DSGCs employ a two-tiered dendritic strategy that enables connections with both ON and OFF starburst cell networks. In recent years, intrinsically photosensitive melanopsin-containing retinal ganglion cells (ipRGCs) have attracted considerable interest in research due to their expression of melanopsin and their intrinsic photosensitivity. ipRGCs represent a unique class of RGCs capable of direct light detection independent of conventional rod and cone photoreceptor signaling. These cells contribute to retinal function through two distinct pathways: they provide excitatory input to dopaminergic amacrine cells and form electrical synapses with GABAergic ACs through gap junctions. These unique properties are believed to play a crucial role in synchronizing the circadian oscillator (Pickard & Sollars, 2012; Qiu et al., 2005).

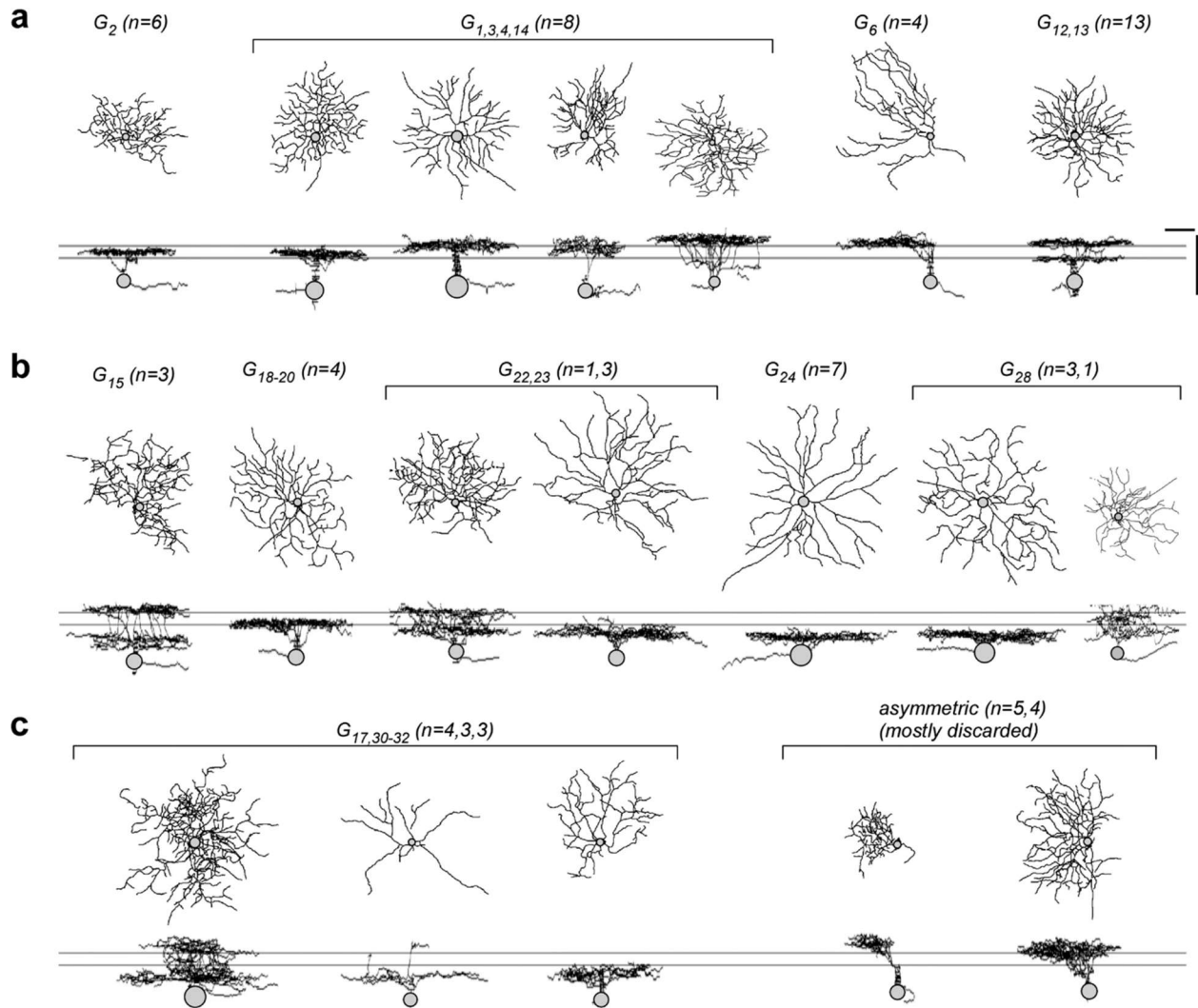


Figure 1.8 Exemplary morphologies of RGCs. Scale bars, 50 μm (Baden et al., 2016). Representative morphologies of RGCs labeled following electrophysiological recording or calcium imaging, then classified into distinct RGC subtypes or excluded based on light response signal-to-noise ratios. Scale bars: 50 μm .

1.2.5.2 Alpha retinal ganglion cells

Among them, Alpha ganglion cells (α RGCs) represent one of the most well-characterized retinal ganglion cell types. Initially identified in the cat retina, these cells are distinguished by their large cell bodies in the RGC layer, large mono-stratified dendritic branches in the IPL, and a robust axon (Boycott & Wässle, 1974; Cleland & Levick, 1974). Based on the response to light, it could also be mainly divided into two kinds of α RGCs (Nelson et al., 1978). There is an ON response in ON α RGC, accompanied by the light on, while an OFF response will appear in OFF α RGC when the light turns off. This property is related to their stratification of dendrites. The dendrites of the ON RGC are close to the ganglion cell bodies. Nevertheless, the dendrites of the OFF RGC are close to the amacrine cell bodies, as shown in **Fig. 1.9** (Krieger et al., 2017).

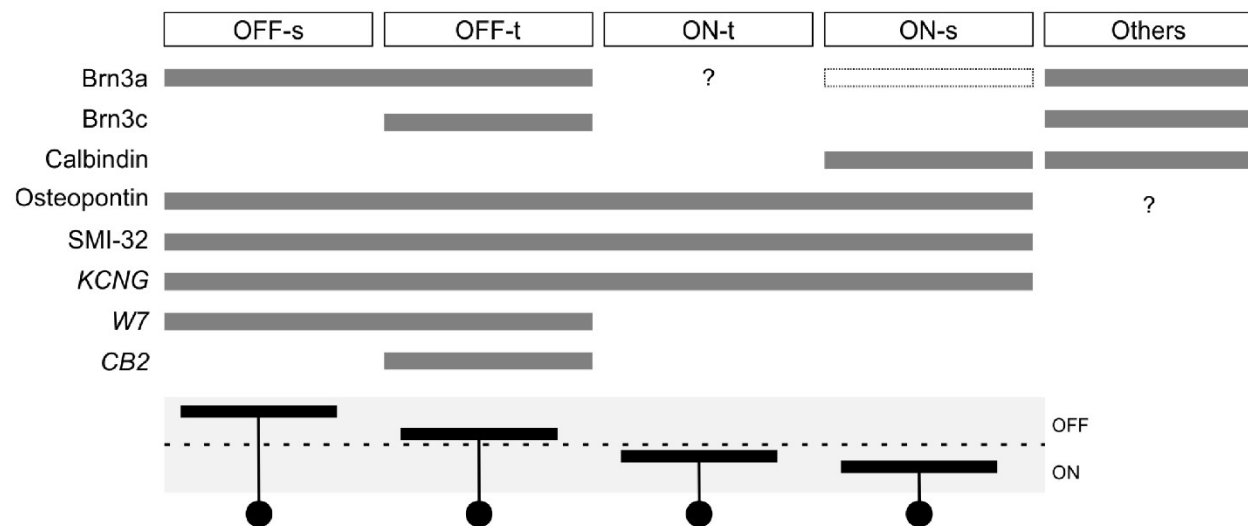


Figure 1.9 A summary of morphological, physiological, and molecular features for the four types of α RGCs (Krieger et al., 2017). Four α RGC variants show distinct IPL dendritic stratification and molecular feature profiles.

A well-established method for objectively evaluating the retinal function is RGC recording. Kuffler was the first person to utilize a microelectrode to detect retinal ganglion cell responses after light stimulation in cats (Kuffler, 1953). Following that, thousands of researchers used and developed RGC recording systems to improve their understanding of retinal circuitry (Takao et al., 2000). Based on the firing characteristics of α RGCs in response to light stimuli, they can be further classified into four types: ON transient, ON sustained, OFF transient, and OFF sustained (Gallego-Ortega et al., 2022; Krieger et al., 2017). A comprehensive electrophysiological characterization of α RGCs in adult mice was conducted, identifying three primary response types: OFF-sustained α RGCs, OFF-transient α RGCs, and ON- α RGCs, with ON responses not further subdivided into sustained or transient categories (Pang et al., 2003). Alpha retinal ganglion cells have been identified in many mammalian species and are commonly labeled by the monoclonal antibody SMI-32 due to their abundance of neurofilaments (Lee & Schmidt, 2018; Tan et al., 2022). Physiological and morphological analyses conducted in C57BL/6J transgenic mice demonstrated that SMI-32+ RGCs exhibit diverse response patterns, including ON-transient, ON-sustained, OFF-transient, and OFF-sustained responses (Krieger et al., 2017). Specifically, mini-alpha RGCs in adult mice display both morphological and immunochemical distinctions from typical α RGCs, featuring smaller soma and dendritic territories while producing transient OFF or ON responses (Baden et al., 2016; Goetz et al., 2022; Ran et al., 2020). However, these cells remain undetectable by SMI-32 antibody labeling (Baden et al., 2016). The choline acetyltransferase (ChAT)-expressing two bands of amacrine cells can be used to distinguish the stratification of four types of α RGCs, which are located in different sublamina of the IPL in the following order from inner to outer: OFF sustained, OFF transient, ON transient, and ON sustained. Anti-osteopontin (OPN)

antibodies enable immunohistochemical identification of α RGCs by targeting this secreted phosphoprotein expressed in these cells (Duan et al., 2015).

ON transient α RGCs exhibit a brief burst of spike activity when the light is turned on, whereas ON sustained α RGCs show maintained spikes throughout the light stimulus period. OFF transient α RGCs are only activated with a brief burst of firing at the end of light exposure, which then quickly diminishes to a minimal level or disappears. OFF sustained α RGCs, also known as noise cells, exhibit maintained spikes in darkness, which decrease or disappear under illumination. The distinct transient and sustained light responses observed in retinal ganglion cells originate from the differential light response properties of their presynaptic transient and sustained bipolar cells. Excitatory drive to two subtypes of OFF cells involves two bipolar cell streams: a sustained bipolar cell pathway dominated by M-cone signals and a transient bipolar cell pathway carrying S-cone-dominated signals (Pang et al., 2003). OFF-transient α RGCs encode looming object size and provide the necessary and sufficient neural signal for looming-evoked fear responses, which represent particularly effective danger-indicating stimuli for animals (Peek & Card, 2016; Wang et al., 2021).

1.2.5.2.1 Inputs of α RGCs

Light-evoked input signals of each type of α GCs are transmitted through distinct combinations of bipolar cells and amacrine cells. the inputs to α GCs in mammals were investigated by recording the light-evoked postsynaptic currents (PSCs) through whole-cell patch-clamp techniques. The PSCs of α RGCs are categorized into excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs), which are recorded by clamping the membrane potential at the

chloride equilibrium potential (E_{Cl}) and the cation equilibrium potential (E_c), respectively. The chloride equilibrium potential (E_{Cl}) is the voltage at which there is no net movement of chloride ions (Cl^-) across the membrane, as the driving force for chloride is zero. By clamping the membrane potential at E_{Cl} , the inhibitory chloride currents are effectively eliminated, which isolates the EPSCs. IPSCs were recorded at the cation equilibrium potential (E_c) because this cancels excitatory cation currents, leaving only inhibitory chloride currents.

ON α RGCs in the mammalian retina received glutamatergic inputs from depolarized bipolar cells and GABAergic–glycinergic inputs from amacrine cells. The differences between transient and sustained ON α RGCs are likely since they are mediated by excitatory inputs to depolarized bipolar cells, which are connected to different types of photoreceptors. Two types of OFF α RGCs exhibit different light responses, which are correlated with different types of hyperpolarized bipolar cells: transient hyperpolarized bipolar cells and sustained hyperpolarized bipolar cells, respectively.

1.2.5.2.2 Outputs of α RGCs

The output signals of RGCs are typically studied by focusing on spikes, which are recorded using the loose-patch technique. Different from the graded potential of photoreceptor and bipolar cells, a spike of a retinal ganglion cell is essentially an action potential, which is subject to the postsynaptic membrane potential. The frequency and pattern of the light-evoked spikes of RGCs carrying abundant visual information were ultimately transmitted to brain nuclei, which encode the light intensity, visual contrast, and RGCs' receptive fields. Short response latencies and fast conducting axons characterize the physiological profile of alpha RGCs (Cleland et al., 1975; Peichl & Wässle, 1981; Wang et al., 2021). Through these properties, α RGCs achieve rapid stimulus

transmission to the brain, positioning them as essential early components of visual processing circuits.

Nearby retinal ganglion cells frequently exhibit concerted activity—the synchronized firing of action potentials at rates significantly above chance levels between two or more neurons (Brivanlou et al., 1998). These cells utilize three distinct correlation mechanisms: broad correlations through shared photoreceptor inputs transmitted via interneuronal chemical synapses, medium-range correlations from amacrine cell excitation through electrical junctions, and narrow correlations from direct electrical coupling between ganglion cells (Brivanlou et al., 1998; Sakai & Naka, 1990; Xin & Bloomfield, 1997). Therefore, electrical coupling in the inner retina emerges as the dominant factor shaping optic nerve firing patterns.

1.3 Synapse in the retina

Retinal synapses can be broadly categorized into two fundamental types—chemical synapses and electrical synapses—which are strategically distributed within two distinct synaptic regions, with different functions in transmission mechanisms.

1.3.1 Chemical synapse

Chemical synapses play an indispensable role in the retina, enabling visual signals to transmit and undergo processing in three distinct pathways originating from photoreceptors. As Samuel Wu effectively summarized, these pathways can be categorized into three functional circuits: the radial pathways, the lateral pathways, and the feedback pathways (Wu, 2010). The retinal network employs a diverse array of neurotransmitters to mediate signal transmission across its complex synaptic architecture. In the radial pathways, glutamate serves as the primary excitatory neurotransmitter, released by photoreceptors and bipolar cells. The lateral pathways utilize inhibitory neurotransmission, with amacrine cells releasing either GABA or glycine to modulate signal spread and feature detection. Horizontal cells employ complex signaling pathways, with GABAergic transmission identified in certain vertebrate species. In the feedback pathways, multiple neurotransmitter systems contribute to diverse synaptic mechanisms, including GABAergic/glycinergic inhibition and dopaminergic modulation.

1.3.2 Electrical synapses

1.3.2.1 Gap junction structure, function, and regulation

Although neuronal communication predominantly occurs through chemical synaptic transmission, the central nervous system (CNS) also utilizes electrical synapses, known as gap junctions, to achieve direct and rapid interneuronal signaling. Gap junctions are intercellular channels formed by two hemichannels in the appositional cell membranes of adjacent cells, which occupy over a 2-4 nm extracellular space. It allows the exchange of ions, molecules, and metabolites between two touched cells. Each hemichannel, also called connexon, comprises six protein subunit connexins, providing conductive channels for membrane-neighboring cells. Gap junction is made of connexin in vertebrates, while formed by innexin in invertebrates (Beyer & Berthoud, 2018). According to the mouse and human gene database, there are 19 kinds of connexin genes in mice and 20 types in humans (Willecke et al., 2002). Connexin protein naming conventions typically combine the "Cx" prefix with numerical values representing the predicted molecular mass of the polypeptide chain in kilodaltons (Beyer et al., 1987). Despite various combinations, only specific connexins are able to form functional hemichannels. Based on the same principle of hemichannel and connexin, a gap junction can also be made of two same hemichannels named “homotypic” and two different hemichannels named “heterotypic”, as shown in **Fig. 1.10** (Bloomfield & Völgyi, 2009).

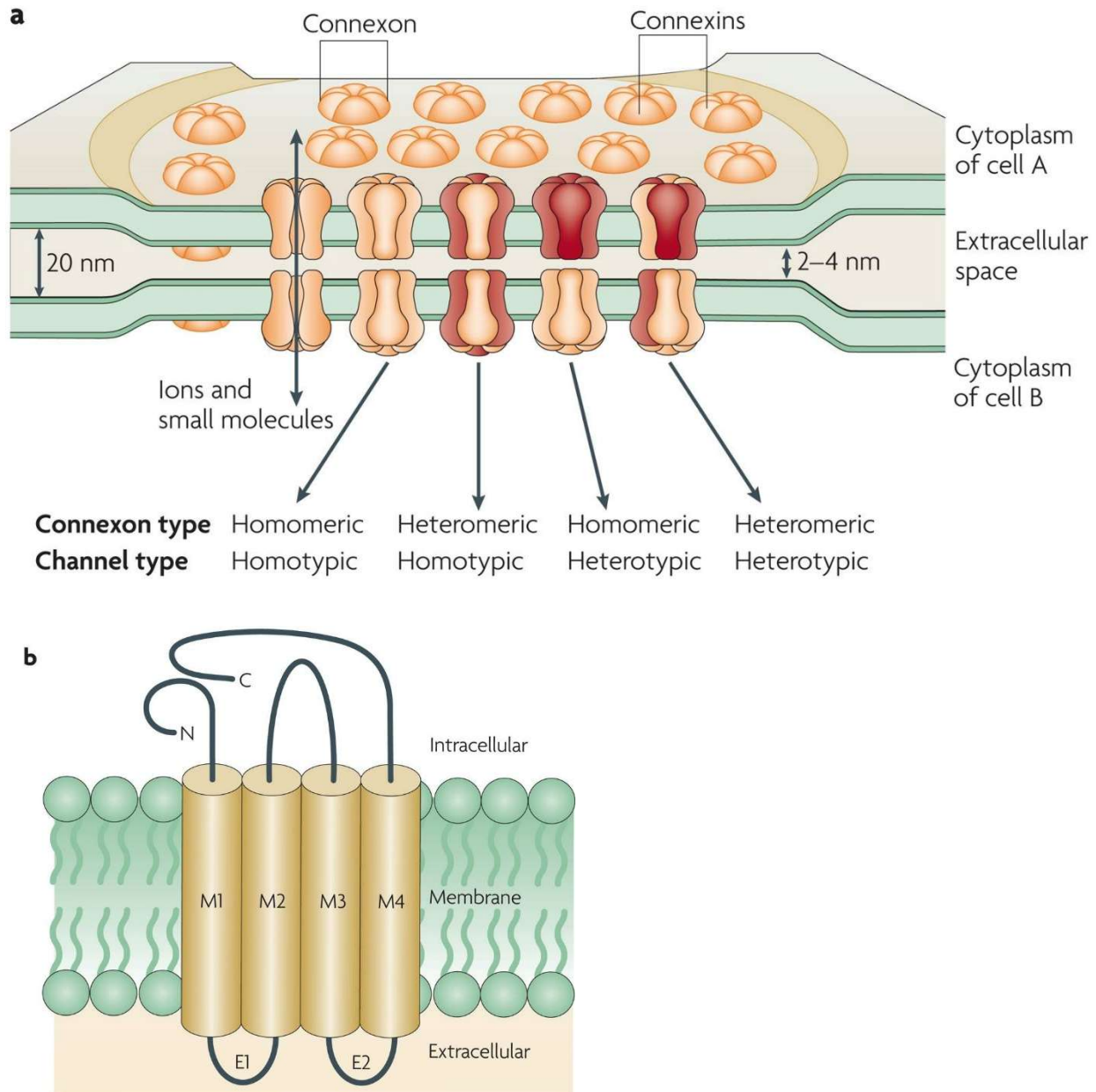


Figure 1.10 Illustration of Gap junctions (a) and connexin subunits (b) (Bloomfield & Völgyi, 2009). (a) Gap junctions are intercellular channels, formed from two HCs on two adjacent cells docking with each other. Each hemichannel comprises six connexin protein subunits, with an aqueous pore in the center. (b) Each connexin subunit consists of four transmembrane segments, two extracellular loops designated E1 and E2, one cytoplasmic loop, and both N- and C-terminal domains located within the cytoplasm.

Early studies assumed that gap junctions are nonspecific passive pores, allowing all ions and molecules up to 1.5 kDa flow (Kirichenko et al., 2021; Simpson et al., 1977). However, recent research proposed the discovery of selective permeability (per selectively), similar to ion channels, indicating that channels are distinct in their conductive function, gating, and permeability (Goldberg et al., 2004). A variety of physiological conditions and substances can influence the conductance of gap junctions (Bloomfield & Völgyi, 2009). Meanwhile, the linkage between diseases and certain connexins has highlighted the uniqueness of each channel, as there is a limited compensation mechanism for the absence of one isoform, which finally results in a pathophysiological deficiency (Bedner et al., 2012; Theis et al., 2003). However, the ion permeability of gap junctions constructed of different connexins shows a slight difference (Nicholson et al., 2000). Gap junctions participate in the transmission of ions and metabolic substances. The former is electrical coupling, producing synchronized and prompt responses in excitable cells such as neurons and the heart, and the latter is metabolic coupling, generating coordinated responses. Therefore, connexins of different intercellular channels have different properties so as to meet the functional requirements of different tissues and organs (Söhl et al., 2004).

Indeed, abundant protein kinases target connexins, and the resulting phosphorylation has been linked to follow-up modulation steps, including trafficking, assembly, and disassembly, and conductive gating of gap junctions (Lampe & Lau, 2000). A variety of variables, including Ca^{2+} -calmodulin, cyclic AMP, cyclic GMP, and neuromodulators, influence these protein kinases. Dopamine (Popova, 1995) and nitric oxide (NO) (Koistinaho et al., 1993) are light-activated neuromodulators generated by amacrine cells, stimulating massive intracellular pathways involving cAMP- and cGMP-dependent protein kinases. This resulting phosphorylation or

dephosphorylation of gap junction connexins modifies their conductance to ionic currents. This modulation in the population of retinal interneurons is controversial: light increases the conductance of some gap junctions while reducing the conductance of others (Bloomfield & Völgyi, 2004; Xin & Bloomfield, 2000). What is more, relying on the light intensity, there is an enhancement or reduction in the gap junction conductance through the same neuron (Bloomfield et al., 1997; Xin & Bloomfield, 1999).

It was reported that the change in intracellular pH probably influences neuronal electrical coupling (Church & Baimbridge, 1991). In general, the conductance of some gap junctions reduces in acidic conditions while increasing in alkaline intracellular conditions. However, the conductance of gap junction connexin36 shows a tendency to increase in alkalinization rather than acidification (González-Nieto et al., 2008). As neural activity shifts the extracellular and intracellular pH, a gap junction between two adjacent cells plays a regulatory role in the generation of activity-dependent pH changes (Chesler, 2003). Besides participating in pH-altering regulated by neural activity, gap junctions appear to be sensitive to voltage differences in the membrane, with the parameters varying depending on the connexin expressed (Srinivas et al., 1999). On the other hand, both voltage-dependent and voltage-independent currents are recorded from single gap junctions (Moreno et al., 1991), which suggests the junctional activity is determined by the cooperation of several conductance sub-states. It also explains why gap junction, unlike action potential, opens and closes in are graded rather than all-or-nothing (D'Souza & Lang, 2020).

1.3.2.2 Neuronal gap junctions in the retina

In the retina, gap junctions serve critical functions in neural signal processing, including noise reduction, signal averaging, neuronal synchronization, and electrical coupling between neurons (Deans et al., 2001; Deans et al., 2002; DeVries et al., 2002). Retinal neural circuits exhibit eight different forms of gap junction-mediated electrical coupling between neurons: Rod-Rod, Rod-Cone, Cone-Cone, Horizontal cell-Horizontal cell, AII amacrine cell-AII amacrine cell, AII amacrine cell-bipolar cell, Retinal ganglion cell-Retinal ganglion cell and Retinal ganglion cell-Amacrine cell, as shown in **Fig. 1.11** (O'Brien & Bloomfield, 2018).

Cx36 is expressed in both hemichannels of gap junctions that mediate electrical coupling between coupled cones and coupled rods (Lee et al., 2003; O'Brien, 2014; Völgyi et al., 2004). Heterocellular coupling occurs between cone-rod pairs, which demonstrates significantly lower strength compared to homocellular rod-rod and cone-cone coupling (Attwell et al., 1984).

Cx57 has been described as being abundantly expressed in the retina, weakly expressed in the thymus, and absent in other tissues of mice (Hombach et al., 2004). In addition, Cx57 is exclusively expressed in mice horizontal cells. Different connexins were expressed in different horizontal cells in the rabbit retina, among which Cx57 is observed in the B-type horizontal cell axon terminal matrix (Pan et al., 2012). Several studies have evidenced that the deficit of Cx57 contributed to the reduced horizontal cell receptive field (Shelley et al., 2006). Notably, Connexin50 (Cx50) is expressed on axonless horizontal cells in the rabbit (O'Brien et al., 2006).

Cx36-mediated coupling between AII amacrine cells involves homologous gap junction proteins, with most homocellular gap junctions displaying linear and symmetrical organization (Deans et al., 2002; Zhang & Wu, 2004). Despite almost 20 connexin proteins capable of forming gap

junctions in mice, IPL neurons selectively express only Cx36, connexin45 (Cx45), and connexin30.2 (Cx30.2) (Güldenagel et al., 2000; Müller et al., 2010). Cx36 localizes to alpha ganglion cell subtypes, while specific bistratified ganglion cells express Cx45 (Hidaka et al., 2004; Pan et al., 2010; Schubert, Maxeiner, et al., 2005; Völgyi et al., 2005). In addition, certain retinal neurons coexpress both proteins, and ultrastructural examination demonstrates that although Cx45 occurs in only 9% of IPL gap junction hemiplaques, these structures consistently colocalize with Cx36 (Li et al., 2008). Gap junction connections between RGCs involve both ACs and other RGCs. Retrograde labeling studies in mice revealed coupling frequencies of 11% for displaced ACs and 4% for conventional ACs with RGCs (Pang et al., 2013). Interestingly, ON α RGCs exclusively couple with displaced ACs, whereas OFF α RGCs establish connections with both conventional ACs and homologous OFF RGCs (Schubert, Degen, et al., 2005; Völgyi et al., 2009). Current evidence suggests that Cx36 forms the gap junction connections between OFF α RGCs and ACs, but the connexin composition of gap junctions in homologous OFF RGC networks and ON RGC coupling systems has not been established (Schubert, Maxeiner, et al., 2005; Völgyi et al., 2005; Völgyi et al., 2013).

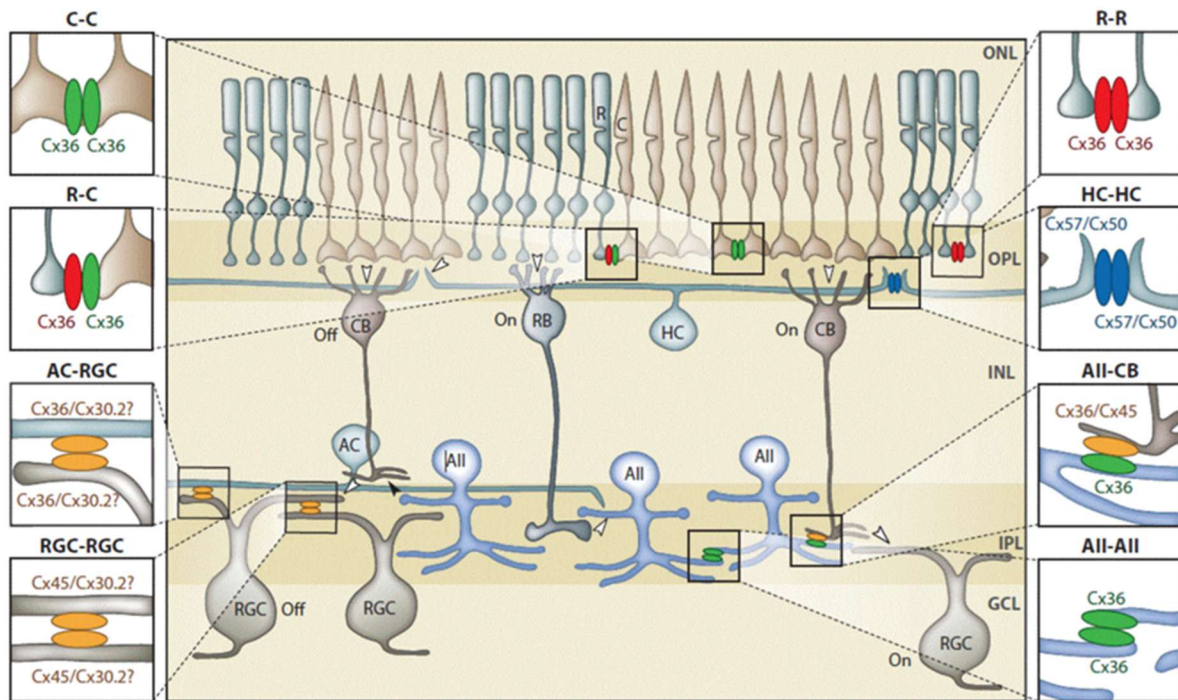


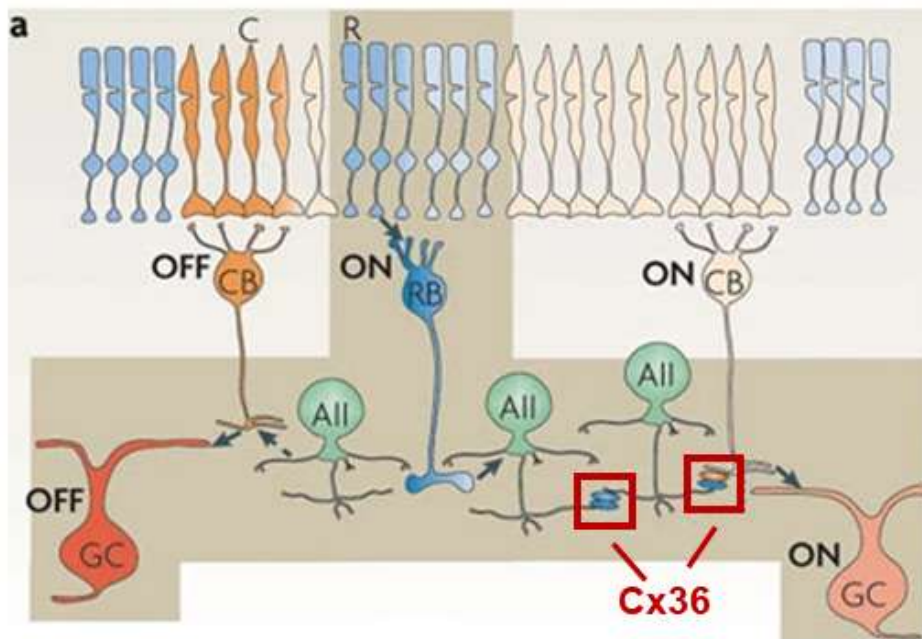
Figure 1.11 Schematic of the electrical circuitry of the retina (O'Brien & Bloomfield, 2018). See details in the text.

1.3.2.3 Connexin36

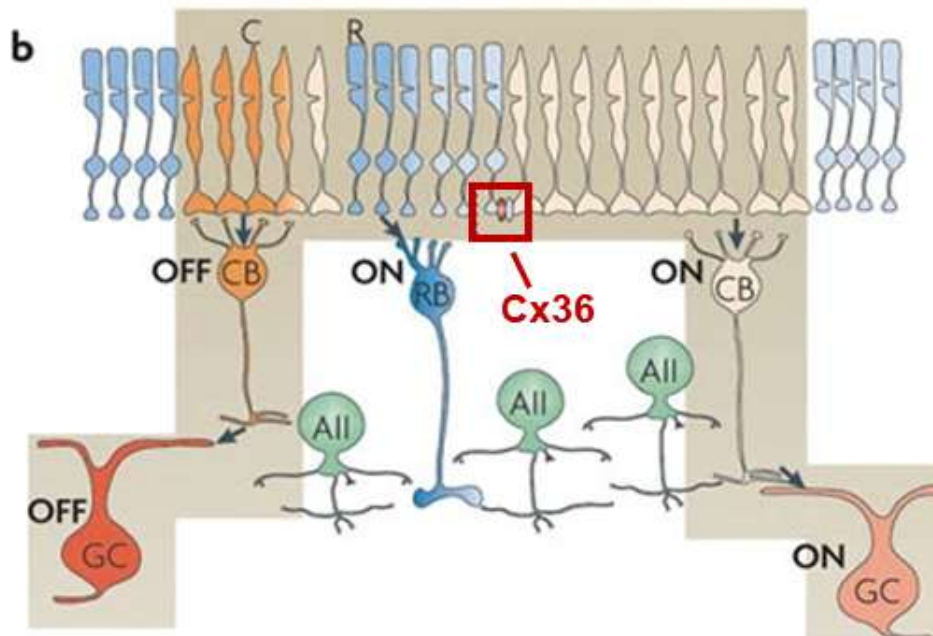
1.3.2.1.1 Connexin36 involved in the visual pathway

Among approximately 20 kinds of connexins in mice, gap junctions in neurons consist of Cx36, Cx45, or Cx57 (Söhl et al., 2005), with Cx36 standing out because of its role in visual pathways. It is considered that retinal Cx36 can achieve noise reduction (Bloomfield & Völgyi, 2009), signal averaging (Söhl et al., 2005), alternative pathways, and a dominant component in the rod pathway, as shown in **Fig. 1.12** (Bloomfield & Völgyi, 2009).

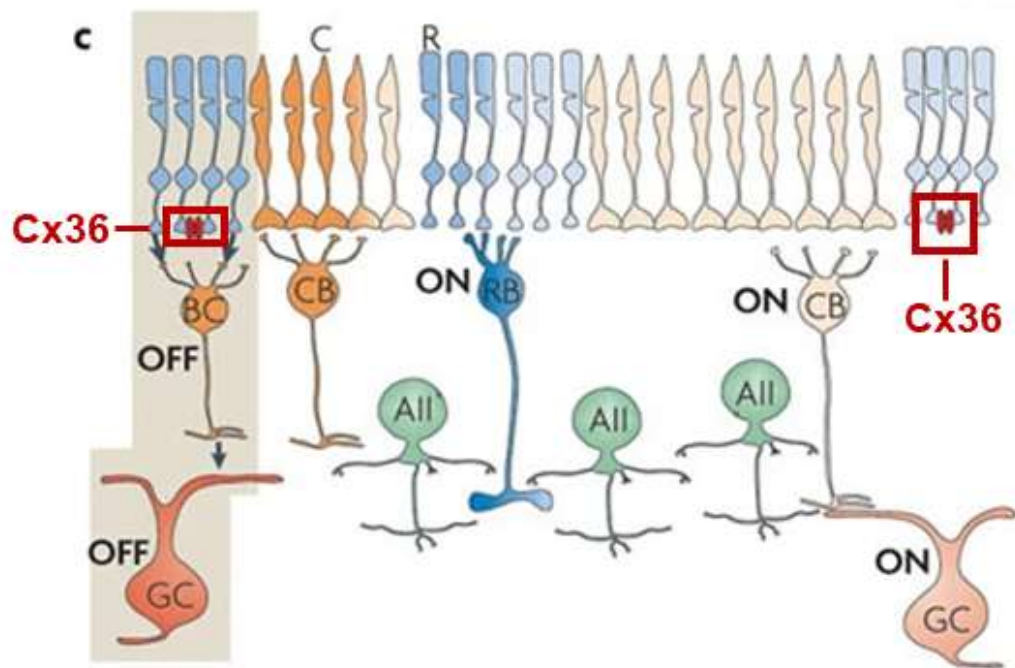
Taking advantage of neuron tracer and punctate immunolabel in the retina, multiple retinal neurons express Cx36 in the cell membrane, including rod photoreceptor, cone bipolar cell, and AII amacrine cell. Taken together, gap junctions are involved between AII amacrine cells and ON cone bipolar cells in the primary rod pathway and between rod and cone cells in the secondary rod pathway, which coincides with the distribution of Cx36 in the retina. Double-label immunofluorescence demonstrated that Cx36 clusters overlap with the matrix of AII amacrine cell dendrites (Feigenspan et al., 2001; Mills et al., 2001). In the Cx36 ablation mice, there is no connection between the AII amacrine cell and the bipolar cell, and all rod signals to ON RGCs are inhibited, with no effect on other ON RGCs' responses (Deans et al., 2002). Both in terms of location and function, it is strongly confirmed that Cx36 plays an essential role in the primary rod pathway. What is more, Cx36 in cone telodendrion is reported to connect with rod spherules (Massey et al., 2003), which implicated the Cx36 gap junction, might be the coupling between cone and rod.



Primary rod pathway



Secondary rod pathway



Tertiary rod pathway

Figure 1.12 Connexin36 in the three rod pathways, modified from (Bloomfield & Völgyi, 2009).

See details in the text.

1.3.2.1.2 Connexin36 involved in secondary retinal cell death

In CNS research, ischemic stroke patients without prompt therapeutic intervention experience a series of deleterious biochemical events termed the "ischemic cascade," which leads to rapid neuronal death not only in the core infarct region but also endangers neurons in a much larger area (Schaller et al., 2003; Schaller et al., 2005). This "secondary degeneration" phenomenon can lead to tissue loss that is more extensive than the primary injury and has also been observed in retinal pathologies (Brunet et al., 2022; Schwartz & Yoles, 1999).

Recently, accumulating evidence demonstrated that Cx36 mediates bystander cell death in the retina. Following the cytochrome C injection into a single RGC, coupled cells show a TUNEL+ reaction, which suggests gap junctions mediate secondary cell death in the retina (Akopian et al., 2014). Compared with wild-type excitotoxic mice, cell survival was enhanced by 50% in Cx36 knock-out mice, which indicated that Cx36 might be involved in regulating secondary cell death (Akopian et al., 2014). Therefore, Cx36 could potentially be involved in neuronal degeneration and regeneration processes. The significance of Cx36 has been highlighted in the secondary cell death rather than primary injury, confirmed by the deletion of the Cx36 protein (Akopian et al., 2017). Previous research suggested that Cx36 deletion and gap junction blockers provided neuroprotective effects to RGCs. The application of MFA (a pharmacological gap junction blocker) or Cx36 gene-deleted mice significantly attenuated the loss of retinal neurons, indicating that connexin36 transfers toxic molecules from apoptotic cells to coupled cells (Akopian et al., 2014; Akopian et al., 2017).

In retinal injury induced by myopia, there is a significant increase in the expression level of the Cx36 protein and phosphorylation (Banerjee et al., 2020; Yang et al., 2019). However, it seems

that Cx36-mediated death only appears in the retina, mainly referring to retinal neurodegeneration diseases. Compared with mice retaining Cx36, Cx36 knock-out mice exhibited no difference in the timing of cone degeneration and the morphological changes in second-order neurons (Kranz et al., 2013), which elucidates that secondary cone death is not altered by the deletion of connexin36 from cones. Research based on glaucoma suggested that up-regulation of retinal Cx36 expression could amplify the degeneration of RGCs, which contributes to further visual function deterioration (Akopian et al., 2017).

1.4 Ocular alkali burn

1.4.1 Pathophysiology and mechanism of anterior segment damage after ocular alkali burn

Alkali burns on the eye could cause permanent, extensive injuries to the ocular anterior segment, mainly the cornea, in unilateral or bilateral vision. Ballen first proposed a grading scheme regarding classifying the severity of the pathophysiology condition after chemical injury (Ballen, 1964), which was adapted by Roper-Hall (Roper-Hall, 1965), which might contribute to the damage level grade and visual prognosis. It was followed by a new grading scheme that involved the combined assessment of limbus, cornea, and conjunctiva (Dua et al., 2001).

Table 1.1 Roper-Hall classification for ocular chemical injury (Roper-Hall, 1965)

Grade	Clock Hours of Limbal Involvement	Conjunctival Involvement	Analog Scale	Prognosis
I	0	0%	0/0%	Very good
II	<3	<30%	0.1–3/1–29.9%	Good
III	>3–6	>30–50%	3.1–6/31–50%	Good
IV	>6–9	>50–75%	6.1–9/51–75%	Good to guarded
V	>9–<12	>75–<100%	9.1–11.9/75.1–99.9%	Guarded to poor
VI	12 (total)	100% (total)	12/100%	Very poor

Table 1.2 Dua classification for ocular chemical injury (Dua et al., 2001)

Grade	Cornea	Conjunctiva	Prognosis
I	Corneal epithelial damage	No limbal ischemia	Good
II	Corneal haze with visible iris details	<1/3 limbal ischemia	Good
III	Total epithelial loss, stromal haze, with obscured iris details	1/3 to 1/2 limbal ischemia	Guarded
IV	Opaque cornea, with obscured iris and pupil	>1/2 limbal ischemia	Poor

Lipophilic alkali compounds permeate cell membranes by saponifying membrane lipids. Hydroxyl ion, which is widely found in alkali compounds, denatures the collagen matrix in the cornea, accelerating more chemicals to penetrate (Soleimani & Naderan, 2020). Liquid necrosis occurs in injured tissues, while proteolytic enzymes will be triggered in response to inflammation, producing a cascade of destruction. In contrast to alkaline substances, acids are generally less destructive as they denature, coagulate, and precipitate corneal proteins on contact, forming a protective barrier against deeper penetration. Strong alkalis can reach the anterior chamber within 15 seconds, injuring the corneal, conjunctival epithelium, basement membrane, stromal keratocytes, endothelium, lens epithelium, episclera, iris, and ciliary body with different degrees of penetration. Penetration might have a sustained effect on the ocular anterior segment after the initial exposure. Direct alkaline exposure of the conjunctiva results in scarring, cicatricial ectropion or entropion, and may lead to dry eye syndrome (Li et al., 2012). Limbal stem cell deficiency caused by chemical injury might tend to generate corneal opacity and neovascularization. Therefore, due to irreversible damage to the cornea or even the entire anterior chamber, patients after alkali burn will experience severe loss of vision and finally lead to blindness. Aimed to rescue vision deterioration brought on by corneal opacity and neovascularization, an abundant number of therapeutic options are utilized in the clinic, mainly concentrating on inflammatory removal and corneal transplantation.

1.4.2 Pathophysiology and mechanism of posterior segment damage after ocular alkali burn

In recent years, increasing cases have been reported in which, despite the cornea recovering transparency after surgery, patients have achieved extremely limited or fruitless improvement in visual parameters, particularly visual acuity. This clinical phenomenon demonstrated that, in addition to the anterior segment, the posterior segment may participate in the progression of impairment following alkali burn. Meanwhile, a significant number of patients experiencing chemical injury suffer glaucomatous complications, with similar results proven in mice and rabbits as well (Paschalis et al., 2018), prompting ophthalmologists and researchers to investigate retinal degeneration following alkali burn injury.

However, the mechanism of ocular posterior segment damage after ocular alkali burn is still unclear. Previous researchers hypothesized that sustained elevated intraocular pressure (IOP) was detected in ocular chemical injury patients with neurodegenerative glaucoma, which might be due to the destruction of the trabecular meshwork and blockade of outflow channels (Lin et al., 2012; Sotozono et al., 1997). Apart from the increased IOP, penetration of chemical components into the anterior and posterior chambers is also a possible trigger for damage. The pH of the aqueous humor had only elevated for 45 minutes, while the measurement by the pH probe of the vitreous was maintained at a normal level (Paschalis et al., 2017). This suggests that there is another pathway to trigger progressive retinal degeneration rather than direct exposure to alkaline substances.

Massive research suggested that most alkali-burnt corneal samples included transcripts for IL-1, TNF- α , and TGF-1 (Planck et al., 1997). Similar experiments on mice also reported that the up-regulated expression of inflammatory cytokines in the iris, ciliary body, and retina, including was

found after 24 hours of burning; the first two including in TNF- α , TNFR-1, TNFR-2, IL-1b, and MMP9, final consisting of TNF- α , IL-1 β , and INF γ (Paschalis et al., 2017). The current perspective also recognizes that retinal neuron damage after alkali burn, featured as retinal ganglion cell apoptosis, which heavily depends on immunological processes that are predominantly mediated by retinal cytokines. Among abundant inflammatory cytokines regulated in the anterior chamber and the posterior segment, TNF- α stands out. The TNF- α function in the physiological condition and pathophysiology of ocular alkali burn is discussed in the following sections.

1.4.3 Cell apoptosis

1.4.3.1 Morphology and significance of cell apoptosis

Morphologically, cellular apoptosis is characterized by a series of sequential changes, including chromatin condensation, DNA fragmentation, membrane blebbing, and culminating in apoptotic body formation.

Following that, macrophages, parenchymal cells, or neoplastic cells phagocytose these bodies. Apoptosis has long been identified as programmed cell death that is normally divided into four phases, from receiving an apoptosis trigger signal to forming an apoptotic body (Elmore, 2007). On the one hand, apoptosis acts as a homeostatic mechanism to maintain cell development and aging. On the other hand, it emerges as a defense mechanism in response to noxious agents evoking immune activity.

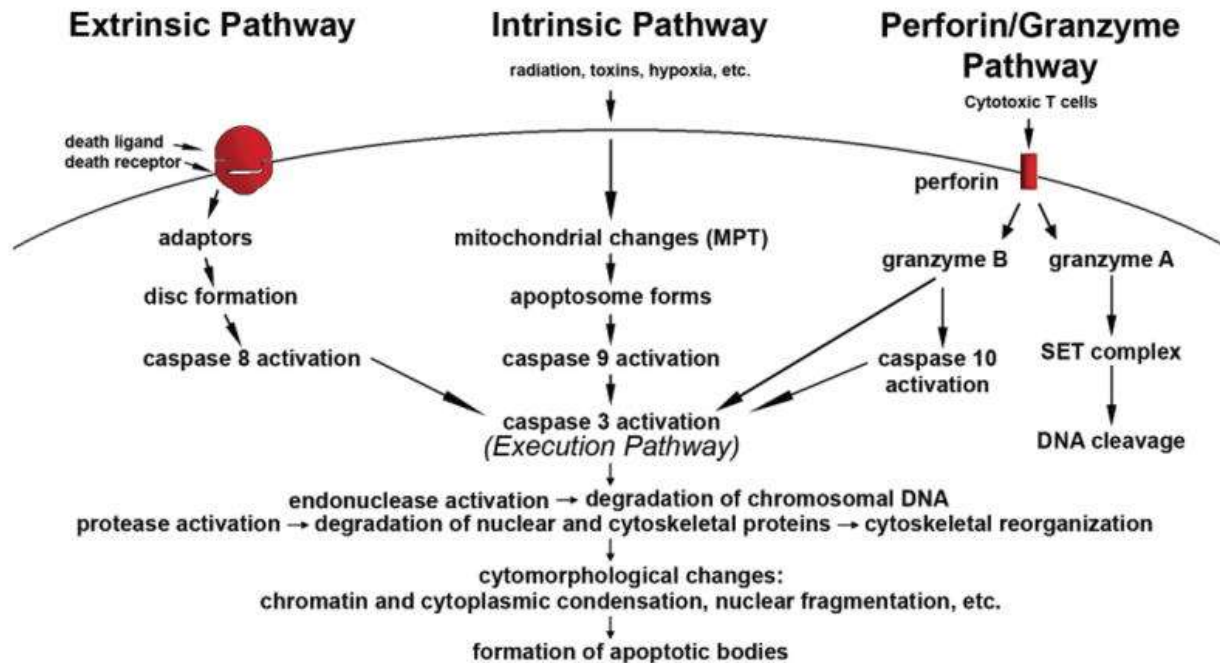


Figure 1.13 Schematic representation of apoptosis, modified from (Elmore, 2007). See details in the text.

1.4.3.2 Role of TNF- α in the cell apoptosis pathway

TNF- α is a type of pro-inflammatory cytokine generated by innate immune cells, like macrophages, microglia, and T-cells, which appear to play a vital role in the inflammatory response and neurodegenerative disorders in the eye (McDermott, 2001; Sharp, 1995; Theodossiadis et al., 2007).

The extrinsic apoptosis pathway is triggered by the ligand of tumor necrosis factor-family death receptors at the cell surface. In classical caspase-dependent extrinsic apoptosis, the TNF- α receptor could recruit the Fas-associated death domain protein in the extracellular environment, which

assembles with pro-caspase-8, thus forming activated caspase-8. Once caspase-8 is activated, it will activate downstream caspase-3 via proteolytic cleavage, finally reaching cell apoptosis (Jan & Chaudhry, 2019).

1.4.4 Apoptosis of retinal ganglion cells in the ocular alkali burn

1.4.4.1 The changes of TNF- α in neurodegenerative diseases

Profound up-modulated expressions of TNF- α and TNF-receptors have been detected in the serum of uveitis patients (Rodrigues et al., 2009). Moreover, the pharmacological blocking of TNF- α contributes to attenuating the progression of retinal cell degeneration (Joussen et al., 2009). In the research based on a 24-hour corneal alkali-burnt animal model, there is a surprising coincidence that the highest point of RGC is accompanied by a remarkable elevation of the level of TNF- α (Cade et al., 2011). What is more, the administration of TNF- α blockers, utilizing a polymer-based drug delivery system, achieved a 4.3% mean reduction of RGCs apoptosis in the rabbit retina after alkali burn (Zhou et al., 2017). Similar results proved that the ablation of TNF- α receptor 1 and 2 contributes to a significant decrease in retinal cell apoptosis in transgenic mice subjected to chemical injury (Paschalis et al., 2017).

Therefore, regardless of directly participating in the caspase-dependent apoptotic pathway or being mediated by an inflammatory response, TNF- α plays a vital role in retinal neurodegeneration after ocular alkali burn.

1.4.4.2 The generation of TNF- α from microglia

TNF- α , a pro-inflammatory cytokine, is rapidly secreted by microglia after CNS and retina injury. It was reported that acutely activated microglia gather primarily in the retinal ganglion cell layer corresponding to the observed retinal damage region in the cornea alkali burn mice mode (Cade et al., 2014). On the one hand, microglia, as a kind of glia cell, play an essential role in maintaining homeostasis away from inflammation out of control via constantly monitoring the environment, searching for tissue injury, or invading microbes. On the other hand, microglia have been identified as a key contributor to retinal degenerative illnesses such as glaucoma and age-related macular degeneration (AMD) (Silverman & Wong, 2018). From a morphological point of view, the homeostatic microglia are predominantly distributed in the retinal outer plexiform layer (OPL), the IPL, and the GCL with ramified shape and small cell bodies, whereas the activated microglia are hard to maintain a branched morphology and can migrate to other layers.

Several lines of evidence showed that microglia play a controversial role in neurodegenerative diseases. For example, the retinal microglia can either offer neurotrophic support to photoreceptors or worsen neuroinflammation and promote the progressive death of neuronal cells. In addition, extensive up-regulated TNF- α and IL-6 produced by microglia reacting to various neuron inflammations are considered as dangerous signals (Ransohoff & Brown, 2012). Recent research declared that the deficiency of retinal gap junctions could inhibit the activation of retinal microglia, ultimately acting as retinal neuroprotection (Kumar et al., 2023).

1.4.4.3 Therapeutic option for retinal ganglion cell apoptosis

Infliximab, a TNF- α inhibitor, is a chimeric IgG1 murine-human antibody. The basic principle of blocking TNF- α is that infliximab can deactivate the biological activity of TNF- α with the combination of strong affinity to the soluble and transmembrane TNF- α , thus inhibiting receptor binding. Consecutive research has demonstrated that the systemic administration of infliximab is widely utilized in rheumatoid arthritis and other autoimmune diseases (Maini & Feldmann, 2002). In addition to systemic administration, topical administration is also widely applied in clinics. There was a remarkable increase in chronic skin ulcers after local treatment with infliximab (Streit et al., 2006). Although the application of infliximab is a viable and feasible therapy, accumulated cases have reported that patients with infliximab appear to be at risk of severe complications, including autoimmune diseases and infections (Lin et al., 2008). Vermeire et al. reported that there were 2 out of 71 Crohn's disease patients who developed lupus erythematosus after infliximab application (Vermeire et al., 2003).

Recently, the multifaceted roles of infliximab in blocking the TNF- α status of the retina have started to be elucidated. Murphy et al. reported that 83.3% of ocular inflammatory disease patients with uveitis, scleritis, idiopathic retinal vasculitis, and ulcerative keratitis achieved clinical remission and significant improvement in immunosuppression during 12 months of mean follow-up with one delayed hypersensitivity reaction (Murphy et al., 2004). Furthermore, in one 7-year follow-up research, symptom remission after infliximab administration, including vitreous haze and retinal vasculitis, was observed in all cases, with almost 50% of eye samples showing visual acuity improvement, and no infliximab-induced complications were reported (Lopez-Gonzalez et al., 2009). Besides being applied in inflammatory diseases, symptoms of neurodegenerative diseases, such as glaucoma, have also improved. The application of TNF- α inhibitor is beneficial

for the protection of retinal ganglion cells in the glaucoma rat model (Roh et al., 2012). It is worth mentioning that, in the treatment of ophthalmological disease patients, infliximab therapy was widely used at the corneal level, such as keratoprosthesis surgery and ocular surface scarring (Ferrari et al., 2013; Robert et al., 2017). In the first mouse ocular alkali burn experiment with infliximab treatment, not only was there a significant decrease in corneal cell apoptosis, but also a remarkable reduction in RGC TUNEL+ cells (Cade et al., 2014), indicating that infliximab plays a protective role in retinal degeneration. This result corresponds with the findings of a subsequent study on alkali burns in rabbits, which showed that systemic administration of infliximab contributes to retinal neuroprotection (Paschalis et al., 2017). Therefore, regardless of corneal or retinal degeneration, prompt administration of infliximab appears to be a feasible therapeutic option, despite potential side effects.

1.5 Glaucoma

Glaucoma is the most common cause of irreversible blindness and will affect more than 100 million individuals by 2040 (Tham et al., 2014). The neurodegenerative disease is characterized by the progressive loss of RGCs and axons, and by degeneration of the optic nerve, ultimately resulting in visual field deficits. Due to the etiologic diversity, the underlying pathophysiological mechanism of glaucoma remains poorly understood. Multiple risk factors contribute to disease development, including gender, myopia, genes, race, and so on (Doucette et al., 2015). Persistent elevation of IOP is the most common risk factor for retinal damage in glaucoma (Asrani et al., 2024). Clinically, reducing IOP is currently also considered the primary therapeutic approach for glaucoma treatment.

Based on the anatomical anterior chamber angle, we can broadly classify glaucoma into two main types: Open-Angle Glaucoma and Angle-Closure Glaucoma. Among them, Primary Open-Angle Glaucoma (POAG) and Primary Angle-Closure Glaucoma (PACG) represent the predominant forms of glaucoma. Since the anterior chamber angle remains open in the former, some glaucoma patients present with normal intraocular pressure symptoms. Genome-wide association studies have confirmed the genomic regions linked with different glaucoma subtypes, including POAG (e.g., *ABCA1*, *AFAP1*), PACG (e.g., *EPDR1*, *CHAT*) (Wiggs & Pasquale, 2017).

1.5.1 Rodent glaucomatous model

Inducible ocular hypertension in experimental animals remains a routine method for deciphering the pathological mechanisms of glaucoma and further exploring novel neuroprotective therapies, with rodents standing out due to their ocular anatomical structure and aqueous humor dynamics closely resembling those of the human eye (Pang & Clark, 2020).

Glaucoma animal models can be broadly categorized into pressure-independent and pressure-dependent models. Pressure-independent models maintain IOP within the normal range, aiming to simulate normal-tension glaucoma while highlighting the contribution of non-IOP-related factors to glaucomatous damage. Representative pressure-independent models include optic nerve crush, retinal ischemia injury, intravitreal injection of excitotoxic amino acids, and intravitreal injection of TNF- α , all of which provide valuable insights into the pathogenesis of glaucoma beyond elevated IOP.

For pressure-dependent models, aside from genetic models that induce varying degrees of IOP elevation, the principle of inducible ocular hypertension models primarily relies on manipulating aqueous humor inflow and outflow. Among these, intracameral injections of various microbeads have been a widely utilized approach, as they reduce aqueous outflow to achieve elevated IOP. In addition, blocking the outflow pathway can also be achieved by inducing damage to the trabecular meshwork (TM) or through anterior chamber cannulation. In recent years, the intracameral injection of silicone oil, as a method to block aqueous inflow via pupillary occlusion, has gained increasing attention from researchers for its application in studies of glaucomatous degeneration (Zhang & Hu, 2020). These approaches provide versatile tools for understanding the underlying

mechanisms of glaucoma and for testing potential therapeutic interventions targeting elevated IOP (Crowston et al., 2015; Kim et al., 2013).

1.5.2 Controversy over the primary site of retinal damage in glaucoma

Although retinal neuron damage is heavily implicated in vision loss in glaucoma, it remains controversial whether the primary injury occurs at the optic nerve head (ONH) or in retinal neurons.

ONH damage has been observed in both the ocular hypertensive mouse model and humans, which was regarded as the primary site of glaucoma damage. In the clinic, this damage is characterized by the appearance of optic disc cupping, resulting in the compression of optic nerve axons, which disrupts the retrograde transport of neurotrophic factors to RGCs, ultimately leading to their death. The different structure of the ONH between acute angle-closure glaucoma and open-angle glaucoma reflects the varying effects of acute IOP elevation and chronic IOP elevation (Kong et al., 2023).

The extensive remodeling of the ONH in human and experimental glaucoma is closely associated with the involvement of activated astrocytes, which are the key cell type at the ONH in most mammalian species. Increased expression of MMPs, TNF- α , and TNF- α receptors has been reported in the glaucomatous ONH, which affects extracellular matrix remodeling (Yan et al., 2000). The total neurite length and axon length of primary RGCs decreased after 72 consecutive hours in an in vitro pressure model set at 50 mmHg, indicating a direct relationship between the elevated IOP and RGCs degeneration (Yan et al., 2000). No significant changes in RGC cell body

area were observed in the three-day in vitro pressure experiment. However, studies conducted in glaucoma rodent models have reported a notable increase in RGC soma area (Ahmed et al., 2001; Kalesnykas et al., 2012). Consistently, similar morphological changes have been identified in glaucoma patients, with an approximately 20% increase in RGC soma diameter compared to control subjects (Liu et al., 2021).

1.5.3 Retinal function in glaucoma

The electroretinogram is an objective, noninvasive tool that sums the responses of all retinal neurons to reflect retinal function. The loss of vision in glaucoma patients has been closely associated with impaired retinal function. Salinas-Navarro et al. investigated retinal function in laser-induced ocular hypertension albino Swiss mice and reported diminished amplitudes of the scotopic threshold response (STR) and a- and b-waves of the flash electroretinogram at the 24-hour time point (Salinas-Navarro et al., 2009).

In addition, Frankfort et al. demonstrated that the decline in normalized positive scotopic threshold response (pSTR) occurred prior to RGC loss, indicating that functional changes in RGCs preceded apoptosis during the early stages of IOP elevation (mean IOP: 15.3 mmHg) (Frankfort et al., 2013). Patch-clamp studies have shown that the lower light sensitivity was recorded in AII ACs, ON and OFF RGCs in elevated IOP mice (15–24 mmHg), not in ON and OFF bipolar cells (Pang et al., 2015). Multielectrode array (MEA) recording performed that the spontaneous activity and receptive field size of OFF transient RGCs decrease after IOP elevation in CD-1 train albino mice and reported that the OFF sublamina are particularly susceptible (Ou et al., 2016). Previous studies

indicate that total synapse loss occurs following IOP elevation; however, there may be compensatory efforts to regenerate immature synapses in the inner retinal layers (Park et al., 2014).

Due to both axons and dendrites being affected by neurodegeneration, the signal transmission from RGCs to the brain might also be altered. It was reported that the resting potential and firing rate of RGCs show a significant increase in glaucoma mice (Risner et al., 2018). The difference in sensitivity between ON and OFF cells can explain why hyper-excitability is found only in OFF cells. Electrophysiological recordings of single RGCs revealed increased spontaneous activity in glaucomatous mice (Ward et al., 2014), although reduced spontaneous activity responses were also recorded. Moreover, the significantly decreased light sensitivity of RGCs occurred after glaucoma induction (Pang et al., 2015).

1.5.4 Piezo channel in glaucoma

Piezo channels are cation-selective, mechanically gated channels consisting of approximately 2,100 amino acids and 24 to 36 transmembrane regions, which are active by mechanical stimuli. There are two subtypes of piezo ion channels: one is Piezo 1 with non-sensory tissues; another is Piezo 2 with sensory tissue. Histology of the mouse eyeball demonstrated that Piezo 1 was expressed on the anterior segment, like the cornea and trabecular meshwork, but also on the posterior segment, especially the RGC and ONH.

The mechanical strain induced by increased IOP has been hypothesized to play an essential role in the pathophysiology of glaucoma, leading to structural and functional damage in RGCs. It has been reported that the upregulated expression of retinal Piezo 1 was found in high IOP retina (Morozumi et al., 2020; Sripinun et al., 2024).

The modulation of Piezo1 activity has been shown to significantly influence neurite outgrowth in RGCs. MEA and patch clamp experiments further demonstrated pressure-sensitive currents and increased spiking activity in RGCs under mechanical pressure, consistent with the effects of Yoda1, an antagonist of Piezo1.

Chapter 2 Retinal Response to Chemical Injury in the Anterior Segment

2.1 Introduction

Ocular alkali burn is a severe emergency that could rapidly cause damage to the ocular surface epithelium, cornea, and anterior segment (Wagoner, 1997). Most ocular chemical burn cases happen in the workplace, especially among young male employees (Quesada et al., 2020). Without prompt assessment and sufficient treatment, the ocular tissues of patients might be likely to continuously suffer from this chemical injury, even leading to severe complications and ultimately blindness (Schrage et al., 2000), which has driven ophthalmologists and researchers to explore the therapy of ocular after alkali burn.

Acid injuries tend to be less severe than alkali injuries. The abundant hydroxyl ion in an alkali solution could deposit into the eyeball surface tissues, thus saponifying the fatty acid of the membrane, causing epithelial cell disruption and death (Ibarguren et al., 2014; Singh et al., 2013). Besides the cornea, alkali compounds can penetrate into the anterior chamber, including the conjunctiva, episclera, iris, and ciliary body. As a result, both short- and long-term therapies are mainly aimed at augmenting the suppression of inflammation and promoting ocular surface re-epithelialization and repair (Baradaran-Rafii et al., 2017). Despite restoring corneal clarity after corneal transplantation, improvement in visual acuity was still disappointing in almost 60% of cases (Westekemper et al., 2017). Meanwhile, one of the common complications found in patients experiencing ocular alkali burn is glaucoma, which contributes to progressive retinal

neurodegeneration (Cade et al., 2011). It has been implied that the retina is involved in the ocular alkali burn damage process. Accumulating evidence suggests retinal neuron damage, characterized as RGC apoptosis, exists in the pathophysiology of ocular alkali burn, which heavily relies on inflammatory responses that are predominantly mediated by retinal cytokine (Miyamoto et al., 1998; Paschalis et al., 2017). Thus, the protection of the retina serves as an undeniably crucial part of ocular alkali burn therapy.

It was reported that the retinal damage induced by corneal alkali burn was not directly mediated by alkali components reaching the posterior segment (Paschalis et al., 2017). Rather, it resulted from the diffusion of massive up-regulation of TNF- α to the ocular posterior segment, finally causing RGC apoptosis (Paschalis et al., 2018). Similar results proved that the peak of RGC apoptosis coincided with a significant elevation of the expression of TNF- α after 24h of ocular alkali burns in mice (Cade et al., 2014), indicating TNF- α as an unignorable player in retinal neurodegeneration after alkali burn. An emerging therapy is infliximab administration, a TNF- α inhibitor, which significantly minimizes retina ganglion cell apoptosis in rabbit models after alkali burn (Zhou et al., 2017). However, this therapeutic option is accompanied by the risk of severe systemic complications (Wong et al., 2008).

Gap junctions are intercellular channels formed by two hemichannels located in the appositional cell membranes of adjacent cells. There are several connexins widely distributed in human and animal retinas, forming conduits to maintain intercellular communication, retinal circuitry, and visual processing, including Cx36, Cx43, Cx45, etc. (Güldenagel et al., 2000; Söhl et al., 2010). Cx36, as a major gap junction protein in the retina, is expressed in most RGC subtypes, especially the α RGC population (Hidaka et al., 2004).

The significance of Cx36 has been highlighted in the secondary cell death rather than primary injury, confirmed by the deletion of the Cx36 protein (Akopian et al., 2017). The application of MFA (a pharmacological gap junction blocker) or Cx36 gene-deleted mice significantly attenuated the loss of retinal neurons, indicating that connexin36 transfers toxic molecules from apoptotic cells to coupled cells (Akopian et al., 2014; Akopian et al., 2017). Research based on glaucoma suggested that upregulation of retinal Cx36 expression could amplify the degeneration of RGCs, contributing to further visual function deterioration (Kumar et al., 2023). These lines of evidence implicate the critical role of Cx36 in the neuroprotection against progressive cell death.

Despite clearer knowledge of Cx36 over the years, there is still limited understanding about the function of Cx36 and its relationship with neurodegenerative diseases in the retina. Considering the neuroprotection function of Cx36 in the progression of glaucoma, we hypothesize that Cx36 also has a similar effect on the pathophysiology of ocular alkali burn. Our findings reveal that Cx36-mediated secondary cell death significantly contributes to the apoptotic degeneration of RGCs following alkali-induced ocular trauma.

2.2 Methodology

2.2.1 Ethics approval

All animal procedures, including feeding, anesthesia, model establishment, and sacrifice, were approved by the Animal Subjects Ethics Sub-Committee of the Hong Kong Polytechnic University (Approval No. 23-24/937-SO-R-GRF). All experiments were followed with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

2.2.2 Animal preparation

2.2.2.1 Mice

All animals were maintained in a 12-h light/12-h dark cycle in the Centralised Animal Facilities in The Hong Kong Polytechnic University. To ensure consistency in the experiment, all mouse enucleation procedures were conducted around 10 a.m. after overnight dark adaptation, and then the retinal tissues were collected immediately following enucleation.

Wild-type C57BL/6J (RRID: IMSR_JAX:000664) (WT) mice of either sex (postnatal days 6–8 weeks old, $n = 93$, 57 male and 36 female mice), weighing (15–25 g) and homozygous Cx36 KO (Cx36 KO HOMO) mice (Cx36^{-/-}) (RRID: MGI: 3,810,172) ($n = 16$, 11 male and 5 female mice, 6–8 weeks old, weight 15–25 g. were used in the experiment. Cx36 KO HOMO mice were identified through genotyping and used for breeding purposes.

2.2.2.2 Genotyping

Tissue from the tip of the mouse tail was collected using scissors between postnatal days 14 and 21 for genotyping. The tail tip was cut into small pieces, placed into a tube, and mixed with 100 μ l of prepared bulk lysis solution (88 μ l dH₂O, 10 μ l KAPA buffer, and 2 μ l enzyme as the sequence). The tubes were placed in a PCR machine (MJ Research PTC-200 Peltier Thermal Cycler) for 15 minutes, and DNA was extracted using the DNA-EX program (75 °C for 10 minutes, 95 °C for 5 minutes, followed by 12 °C hold).

After DNA extraction, the tubes were centrifuged at 10,000 rpm for 2 minutes to collect the supernatant, which was used as the DNA sample, and a 25 μ l reaction mixture was then prepared by sequentially combining 12.5 μ l of Taq polymerase (Taq DNA polymerase, hot start, Talara Cat#R028A), 8.5 μ l of dH₂O, 0.5 μ l of each primer, and 2 μ l of the DNA sample. After thoroughly mixing the solution by shaking, the tubes were placed into the PCR machine to run the designated PCR program for 2 hours.

To prepare the agarose gel, 2.7 g of agarose was added to 150 ml of 1×TAE buffer, and the mixture was microwaved in 30-second intervals until the agarose was completely dissolved in the TAE solution. Once dissolved, the solution was allowed to cool to approximately 60°C, after which 7.5 μ l of 20,000× dye was gently added to the bottle and mixed carefully to avoid the formation of bubbles. Finally, the solution was poured into a gel caster with the comb properly positioned, ensuring the gel was as even as possible.

A 5 μ l DNA ladder was added to both sides of the gel, and 20 μ l of PCR products were mixed with 5 μ l of loading buffer at a ratio of 1:5 before being carefully loaded into the wells of the gel. After ensuring the samples were properly placed, the lid of the electrophoresis chamber was closed,

paying attention to the orientation of the positive and negative poles. Finally, the power supply was turned on, and the voltage (90V) and runtime (1h) were set for the electrophoresis process. The results of genotyping were obtained by exposing the gel to UV light for visualization.

2.2.2.3 Cornea alkali burn mouse model

The cornea alkali burn mouse model was established according to the method. Mice were deeply anesthetized via intraperitoneal injection of the mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg). Subsequently, a drop of lidocaine (20 mg/ml) was applied locally to the surface of the eyeballs, including the cornea, eyelids, and surrounding tissues to provide topical analgesia and anesthesia. A toe pinch test will be used to confirm whether the mice are in deep anesthesia before proceeding with the next step of the experiment. In this experiment, only the right eye of each mouse was used. A 2-mm-diameter filter paper would be prepared in advance, soaked in 1 mol/L sodium hydroxide solution for 10 seconds, and then placed on the mouse central cornea. Subsequently, 2 μ l of 1 mol/L sodium hydroxide solution was applied to the filter paper using a micropipette and maintained for 30 seconds. After removing the filter paper, the cornea was promptly irrigated with sterile saline for 20 seconds. The mouse was then placed laterally on a 37 °C heating pad and monitored until fully awake.



Figure 2.2.2.3 Illustration of mouse cornea following alkali-induced injury.

2.2.3 Intravitreal injection

The mice were deeply anesthetized for systemic anesthesia using an intraperitoneal injection of a ketamine/xylazine mixed solution at a dose of 80 mg/kg:10 mg/kg body weight. Lidocaine hydrochloride (20 mg/ml) eye drops were applied to anesthetize the cornea and surrounding tissues of the mice.

A disposable intravenous infusion tube was used to connect a homemade borosilicate glass capillary micropipette to a medical syringe. Prior to the procedure, 2 μ l of the gap junction blocker meclofenamic acid (MFA) at a concentration of 200 μ M was carefully filled into the homemade micropipette. A surgical blade was used to create an initial incision below the ora serrata, after which the tip of the micropipette was quickly inserted into the incision. The syringe was then gently pressed to allow the MFA in the micropipette tip to be injected into the vitreous cavity of

the mouse eye. After the injection was completed, the micropipette tip was left in the eyeball for 30 seconds before being carefully withdrawn. After the procedure, the mice were placed on a 37°C heating pad to recover from anesthesia.

The same procedure was applied for the intravitreal injection of HEPES solution at a dosage of 1.5 µl with a concentration of 0.1 M.

2.2.4 Retina preparation

The experiment was conducted under dim red illumination to maximally preserve the light sensitivity of dark-adapted tissues. Following enucleation, intact eyeballs were immediately transferred to fresh bicarb solution to maintain cellular viability. A surgical blade was used to create an initial incision along the ora serrata, after which ophthalmic scissors were employed to separate the anterior segment from the posterior segment of the whole eye. The retina was subsequently extracted from the posterior segment for experimental use, and the vitreous humor was carefully removed using ophthalmic surgical forceps to prevent interference with microscopic observation of retinal neurons.

The cup-shaped retina was cut into small pieces using ophthalmic scissors. These pieces were then placed on a specialized filter, where negative pressure from a suction device was applied to secure them firmly in place. The filter containing the retinal pieces was subsequently transferred into a pre-prepared chamber. The chamber was filled with continuously perfused Ringer's solution. This setup simultaneously provided the retinal pieces with 32 °C and 95% O₂/5% CO₂ to maintain cellular viability.

Mouse bicarb solution is prepared by combining NaCl (3.656 g), KCl (0.093 g), NaH₂PO₄ (0.075 g), D-glucose (1.801 g), and NaHCO₃ (1.902 g), which are weighed out and completely dissolved in 500 ml of ddH₂O through continuous stirring for 15 minutes. Following complete dissolution, the solution is bubbled with 95% O₂/5% CO₂ for 10 minutes at room temperature to establish proper oxygenation and carbonic acid buffering. Pre-prepared MgCl₂ solution (500 µl) and CaCl₂ solution (1000 µl) are then added sequentially, followed by an additional 10-minute dissolution period with continuous stirring to ensure complete ionic integration. The pH is subsequently adjusted to 7.4 using a calibrated pH meter, and the final bicarb solution is ready for experimental use. To maintain optimal physiological conditions and prevent degradation of buffer components, a fresh bicarb solution should be prepared every three days and stored under appropriate conditions between uses.

Mouse Ringer's solution preparation begins by weighing NaCl (7.013 g), KCl (0.373 g), NaHCO₃ (2.1 g), Na₂HPO₄ (0.114 g), NaH₂PO₄ (0.012 g), and D-glucose (1.802 g), which are then completely dissolved in 1000ml of dH₂O through continuous stirring for 15 minutes at room temperature. The solution is subsequently oxygenated by bubbling with 95% O₂/5% CO₂ for 10 minutes to establish proper gas equilibration and buffering capacity. Following oxygenation, MgSO₄ (0.247 g), CaCl₂ (0.294 g), and L-ascorbic acid (0.018 g) are weighed and added to the solution, requiring an additional 15 minutes of stirring to achieve complete dissolution and uniform distribution of all ionic components. The final pH adjustment to 7.4 is performed using a calibrated pH meter to ensure physiological compatibility. To maintain optimal neuronal viability and prevent degradation of sensitive components, particularly L-ascorbic acid, fresh Ringer's solution should be prepared twice weekly, ensuring that experimental conditions consistently support maximal

preservation of neuronal cell integrity and functionality throughout electrophysiological recordings.

2.2.5 Patch clamp

2.2.5.1 Intracellular dye injections

A mixture of 4% Neurobiotin tracer (Vector Laboratories, Burlingame, CA, USA) and 0.5% Lucifer Yellow-CH (Molecular Probes, Eugene) was filled from the back end of the microelectrode while observing it gradually spread the entire sharp pipette tip under 10× microscopic magnification. After double-filtering the intracellular solution, the micropipette was filled with the intracellular solution. The electrode tip is positioned close to the target neuron, and the membrane is gently punctured to minimize cellular damage, allowing the dye to diffuse into the cell. When necessary, pulse or turning modes can be used to improve diffusion efficiency and accelerate dye entry into the cell. The mixed tracer was injected iontophoretically using a sinusoidal current (5 Hz, 5 nA p-p). The fluorescence can be activated to verify successful cellular injections by visualizing the Lucifer Yellow fluorescence within the neuron under 40× microscopic magnification.

After completing the dye injection, quickly withdraw the sharp electrode from the cell. During this process, be careful not to damage the cell structure and avoid a situation where the electrode pulls the cell away. Then, remove the filter with the retina piece from the workstation and place it in freshly prepared 4% PFA for fixation. To obtain coupled cells, the filter with the retina piece needs to be placed in an environment maintained at 32 °C with 95% O₂ /5% CO₂ for 15 minutes, followed by 4% PFA fixation. To protect the fluorescence, the fixation process should be carried out in the

dark. For neuronal identification, Brn3a immunostaining will be employed to specifically identify RGCs, while SMI32 labeling will serve to distinguish α RGCs.

2.2.5.2 Patch-clamp recording

Following cellular seal and configuration, the minute cellular signals were amplified and detected through a MultiClamp 700B amplifier. The amplified signals were then digitized by the Digidata 1550B interface, converting the electrical signals into digital signals (A/D conversion). In experiments, converting digital signals back into electrical signals is also commonly employed (D/A conversion). A computer was connected to the interface to record and process real-time digital signals. The pCLAMP 10 software (Molecular Devices) package includes Clampex for data acquisition and Clampfit for analysis. Microelectrodes were pulled to 3-7 Ω resistance and filled with internal solutions.

In patch clamp recording, the loose patch technique and the whole cell technique are performed as follows:

Loose patch technique: The microelectrode is gently positioned near the targeted cell surface. Mild negative pressure is applied on the surface of the cell membrane, forming a low-resistance (several M Ω) seal. Throughout the experiment, the cell membrane remains intact, with the cell maintaining its complete morphology.

Whole cell technique: Relatively strong negative pressure is applied to the target cell membrane to form a high resistance gigaohm seal, followed by applying brief electrical pulses to break the cell membrane under the seal, allowing direct communication between the electrode solution and the cytoplasm.

The target tissue in the chamber was observed under near-infrared light (>775 nm). After observing the retina piece under 10x magnification, target neurons were examined in detail using 40x magnification. A fixed-stage microscope (Eclipse FN1; Nikon, Tokyo, Japan) equipped with differential interference optics and a Nikon tube camera (Dage-MTI, Michigan City, IN) was utilized for cellular visualization. To prevent high flow rates of perfusion solution from affecting cellular recordings, the Ringer's solution perfusing the retinas was controlled at a rate of 1-1.5 mL/min.

Spike trains were recorded using Axoscope software at a standard sampling rate of 10 kHz to capture the firing pattern and timing accurately. The acquired spike data were sorted by the Off-line Sorter software (Plexon, Dallas, TX), followed by analysis and statistical processing with NeuroExplorer (Nex Technologies, Littleton, MA). The recorded cells were identified by dye injection to confirm cell type classification.

2.2.5.3 Light stimulation

A green light-emitting diode (LED) with a wavelength of 525 nm was positioned adjacent to the recording setup to deliver uniform full-field stimulation to the isolated retinal preparation. The light intensities were measured using the ILT5000 radiometer (International Light, Peabody, MA) in milliwatts per square centimeter. In visual physiology, these measurements were converted to the rate of photo-isomerizations per second ($Rh^*/rod/s$), representing the number of chemical changes in rhodopsin molecules occurring in retinal photoreceptors (rods) due to light stimulation. In calculating light intensity, an average rod density of 437,000 rods/mm² and quantum efficiency of 0.67 were used.

The light intensity–response relationship for individual cells was calculated by collecting spike counts and current amplitudes 1 second before, during, or after stimulation, with light intensities ranging across 5 log units from 2.9×10^1 to 1.2×10^5 Rh*/rod/sec.

The ON responses of ON α RGCs and OFF responses of OFF α RGCs were recorded, with spike counts for ON and OFF responses calculated after subtracting background or spontaneous spikes. Cells were classified as transient or sustained based on the characteristics of their light-evoked spike responses at light onset and offset. The number of light-evoked spikes was normalized on a cell-by-cell basis. The sensitivity curves, which were generated by plotting normalized light-evoked responses against light intensities using Python software, were fitted using the Hill equation.

$$R/R_{max} = I^N / (I^N + \sigma^N)$$

$$= 0.5[1 + \tanh 1.15N(\text{Log } I - \text{Log } \sigma)]$$

R/R_{max} is the normalization method for light-evoked responses, where R represents the spike count or current response amplitude, and R_{max} corresponds to the maximum spike count or response amplitude. N is the Hill coefficient, and σ is the light intensity required to evoke a half-maximal response. The sensitivity response threshold is defined as the light intensity that elicits 5% of the maximal spike count or current response amplitude.

2.2.6 Immunohistochemistry

2.2.6.1 Whole mount for mouse retina

The eyeballs of the mice were removed after anesthesia and placed in a fresh bicarbonate solution. Subsequently, surgical blades, ophthalmic scissors, and forceps were used to extract the retinas quickly. The retinas were then cut into a clover shape using ophthalmic scissors and transferred onto a filter, which effectively stabilized their structure and prevented curling during subsequent experiments.

The filter, along with the attached retina, was placed in 4% paraformaldehyde solution, which was freshly prepared weekly, and fixed for 20 minutes. After fixation, the retina was carefully and gently peeled off the filter using a brush. The retina was then rinsed with rinse solution every hour for a total of six times. After rinsing, the retina was blocked with 3% donkey serum solution and stored overnight at 4°C. Following the blocking step, the retina was placed in a tube containing a mixture of the primary antibody and 1% donkey serum for staining, which lasted for one week. After one week, the retina was removed and rinsed with rinse solution every hour for a total of six times to remove the excess primary antibody. Subsequently, the retina was placed in a tube containing a mixture of the secondary antibody and rinse solution and stored overnight at 4°C. On the following day, the retina was rinsed again every hour for six times. For mounting, the retina was carefully transferred onto a glass slide with the GCL facing upward. Before placing the coverslip, a drop of mounting medium was added, completing the mounting process. The primary antibodies used in this study were listed in **Table 2.1**. The secondary antibodies were Alexa Fluor 633 anti-Goat IgG (Thermo Fisher Scientific, Cat#A-21082, 1:200), 488 anti-mouse IgG

(Molecular Probes, Cat# A-21202, 1:200), Streptavidin-Cy3 from Streptomyces avidin (Merck, Cat# S6402-1ML, 1:200), 488 anti-rabbit IgG (Thermo Fisher Scientific Cat# A-21206, 1:200)

Table 2.1 Antibodies used in the studies

Antibody	Host	Company; Cat#	Concentration
Anti-Choline Acetyltransferase antibody	Goat	Millipore; Cat# AB144P	1: 200
Anti-Brn-3a antibody	Mouse	Santa Cruz; Cat#SC8426	1: 200
Anti Iba1	Rabbit	FUJIFILM Wako Pure Chemical Corporation; Cat# 019-19741	1:500
Purified anti-Neurofilament H (NF- H), Nonphosphorylated Antibody (SMI-32)	Mouse	BioLegend; Cat# 801701	1:200

2.2.6.2 TUNEL labeling for mouse retina

Following the alkali burns, the whole-mount retinas were prepared. Apoptotic cells were detected using TUNEL (Tdt-mediated dUTP-X-nick end labeling) with the In Situ Cell Death Detection Kit, TMR red (Roche, Basel, Switzerland). TUNEL was added according to the manufacturer's instructions. 0.1% Triton X-100* in 0.1% sodium citrate was used as the permeabilization solution. Subsequently, the retina was rinsed twice with PBS at room temperature.

The TUNEL reaction mixture was freshly prepared immediately before use by combining Enzyme Solution and Label Solution at a 9:1 ratio (v/v). The mixture was thoroughly vortexed and kept on ice until application to prevent degradation of enzymatic components. The TUNEL reaction mixture was then added onto the retina and incubated for 60 minutes at 37°C in a humidified atmosphere in the dark. Following incubation, the retina was rinsed three times in PBS for 5 minutes each. Before slide mounting, 4,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, US, D9542) was added.

2.2.7 Imaging acquisition and processing

Images of the retinal whole were acquired using a ZEISS LSM 800 confocal microscope with Airyscan (Zeiss, Thornwood, NY, USA), equipped with 20× and 40× objectives. For whole-mount, the mouse retina was divided into three regions: the far-peripheral area (1700 µm from the optic nerve), the mid-peripheral area (1100 µm from the optic nerve), and the central area (500 µm from the optic nerve). From each region, four images were selected, yielding a total of twelve images per mouse for retinal cell counting. The images were processed and analyzed by using ImageJ software (ImageJ, an open source developed by National Institutes of Health, Bethesda, MD, USA,

1.54 g, RRID: nif-0000- 30467). The percentage was calculated by dividing the number of Brn3a/TUNEL double-labeled cells by the total number of Brn3a-positive cells.

Single RGC morphology was imaged using a ZEISS LSM 800 confocal microscope with Airyscan technology. Somatic and dendritic field areas were automatically quantified using ZEISS software, with diameters calculated from equivalent circle measurements.

All data were presented as mean $\pm$ SEM. Comparisons between the two groups were analyzed using an unpaired t-test, whereas comparisons among the three groups across different regions were analyzed using a one-way analysis of variance followed by Tukey's multiple comparison test. Statistical analysis was performed using commercial statistical software (Prism 9.5.1 [RRID: SCR_002798]; GraphPad, Inc., La Jolla, CA, USA). The P-value notation used for statistical analysis is shown in **Table 2.2**.

Table 2.2 The statistical significance symbols used in the analysis

symbols	<i>P</i> -value
ns	$p > 0.05$
*	$0.05 > p > 0.01$
**	$0.01 > p > 0.001$
***	$p < 0.001$

2.3 Results

2.3.1 Corneal alkali burns result in significant death of retinal ganglion cells

To elucidate the effects of alkali-induced corneal injury on retinal neurons, we examined the onset and progression of RGC apoptosis within the initial 24 hours post-injury using Brn3a as a marker for RGCs and the TUNEL assay to identify apoptotic cells in WT mice. We specifically selected four critical timepoints for analysis: 4, 6, 12, and 24 hours post-injury, comparing cell quantities between control eyes and those subjected to corneal alkali burns. The results revealed a significant temporal and spatial progression of TUNEL-positive RGCs following corneal alkali burn, with the apoptotic process beginning in the far-peripheral retina and gradually extending to the mid-peripheral and central regions over time across these sequential timepoints.

At 4 hours post-injury, TUNEL-positive reactions were predominantly localized to the retinal periphery (**Fig. 2.3.1A**). Quantitative analysis (**Fig. 2.3.1B**) revealed distinct regional differences in RGC apoptosis percentages. The far-peripheral area demonstrated the highest proportion of TUNEL-positive RGCs (0.58 ± 0.03 , mean $\pm$ SEM, $n = 8$), which was significantly elevated compared to both the mid-peripheral (0.07 ± 0.01 , $n = 8$) and central regions (0.04 ± 0.01 , $n = 8$). By 6 hours post-injury, TUNEL-positive reactions began to appear in the equatorial region while remaining predominantly concentrated in the retinal periphery (**Fig. 2.3.1A**). Apoptosis (**Fig. 2.3.1C**) had increased progressively across all regions, with the far-peripheral area exhibiting 0.90 ± 0.02 ($n = 8$), the mid-peripheral region showing substantial elevation to 0.53 ± 0.04 ($n = 8$), while the central area remained relatively unaffected at 0.05 ± 0.01 ($n = 8$). At 12 hours post-injury,

TUNEL-positive reactions had extended into the central region, while the majority of apoptotic activity remained concentrated in the peripheral areas (**Fig. 2.3.1A**). RGC apoptosis (**Fig. 2.3.1D**) reached peak levels in both the far-peripheral (0.96 ± 0.01 , $n = 8$) and mid-peripheral regions (0.92 ± 0.01 , $n = 8$), while the central region demonstrated a significant but more modest increase to 0.13 ± 0.02 ($n = 8$). By 24 hours post-injury, apoptosis spread throughout the entire retina (**Fig. 2.3.1A**), with consistently high levels across all regions (**Fig. 2.3.1E**): far-peripheral area at 0.95 ± 0.01 ($n = 8$), mid-peripheral at 0.96 ± 0.01 ($n = 8$), and central area at 0.96 ± 0.02 ($n = 8$).

Cell density measurements of TUNEL/Brn3a double-positive cells confirmed the peripheral-to-central progression pattern observed in percentage analysis. At 4 hours post-injury, apoptotic RGCs (**Fig. 2.3.1F**) showed the highest density in the far-peripheral area (1714 ± 137 cells/mm², $n = 8$), followed by the mid-peripheral area (253 ± 24 cells/mm², $n = 8$), and the lowest density in the central region (154 ± 18 cells/mm², $n = 8$). By 6 hours, cell density (**Fig. 2.3.1G**) had increased substantially across all regions, with TUNEL/Brn3a double-positive cells demonstrating the highest density in the far-peripheral area (2626 ± 124 cells/mm², $n = 8$), intermediate levels in the mid-peripheral region (1889 ± 128 cells/mm², $n = 8$), and minimal involvement in the central area (189 ± 11 cells/mm², $n = 8$). At 12 hours post-injury, density measurements (**Fig. 2.3.1H**) revealed similarly high concentrations in both the mid-peripheral (2674 ± 165 cells/mm², $n = 8$) and far-peripheral regions (2468 ± 112 cells/mm², $n = 8$), while the central area exhibited significantly lower density (416 ± 111 cells/mm², $n = 8$). Twenty-four hours after injury, the density of TUNEL/Brn3a double-positive cells had reached comparably high levels across all retinal regions: central areas showing 2926 ± 277 cells/mm² ($n = 8$), mid-peripheral regions demonstrating 2906 ± 199 cells/mm² ($n = 8$), and far-peripheral areas exhibiting 3035 ± 281 cells/mm² ($n = 8$) (**Fig. 2.3.1I**).

These findings reveal a distinct spatiotemporal pattern of RGC apoptosis following a corneal alkali burn, starting in the far-peripheral retina and progressively moving toward the central retina over 24 hours. This pattern suggests a potential wave of retinal damage spreading from the periphery inward, possibly due to the diffusion of inflammatory mediators or other injury-related signals from the cornea to the retina.

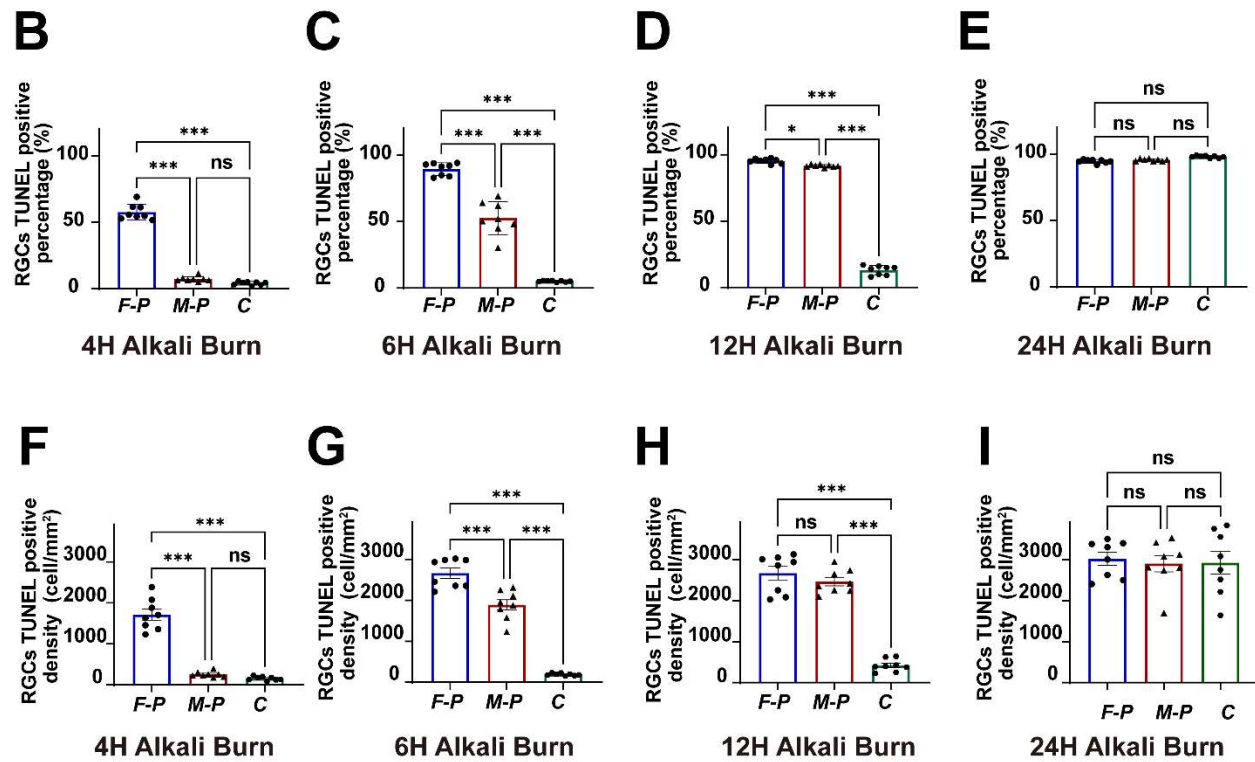
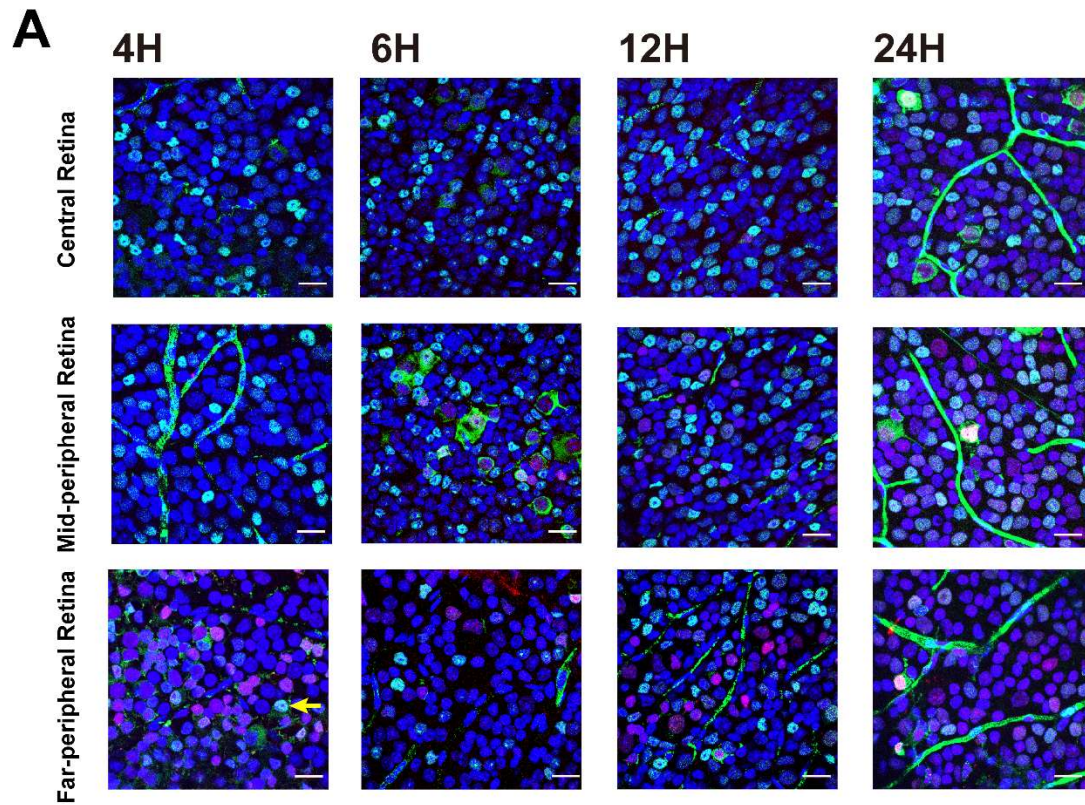


Figure 2.3.1 The apoptosis of retinal ganglion cells resulting from corneal alkali burns.

(A) Representative confocal images of retinal whole-mounts from WT mice taken at 4, 6, 12, and 24 hours after corneal alkali burn (green = Brn3a; red = TUNEL; and blue = DAPI). A representative Brn3a/TUNEL double-positive cell is indicated by the yellow arrow.

The percentage of TUNEL-positive nuclei in the RGC layer across three retinal regions—far-peripheral (F-P), mid-peripheral (M-P), and central (C) at 4 hours (B), 6 hours (C), 12 hours (D), and 24 hours (E) post-alkali burn.

The density of TUNEL/Brn3a double-positive cells was assessed in three retinal regions (F-P, M-P, and C) at 4 hours (F), 6 hours (G), 12 hours (H), and 24 hours (I) following alkali burn. Statistical analysis was performed using 1-way ANOVA followed by Tukey's multiple comparison test. *Scale bars* represent 20 μm .

2.3.2 Gap junction-mediated apoptosis in retina

We conducted a replication study examining gap junction-mediated cell death in α RGCs and their coupled neuronal cells using whole-mount retinal preparations (**Fig. 2.3.2**). α RGCs received co-injections of Neurobiotin and cytochrome C, allowing simultaneous visualization of gap junction coupling and induction of apoptotic cell death in coupled neuronal cells. TUNEL staining confirmed apoptotic changes in the neuronal cells that were gap junction-coupled to the injected α RGCs. These findings provide evidence that apoptotic signals can propagate from α RGCs to neighboring neuronal cells via gap junction pathways. Our dose-response analysis revealed that cytochrome C at 10 mg/mL concentration effectively triggers secondary neuronal death in α RGCs,

while the 1 mg/mL concentration showed reduced efficacy. These results indicated a dose-dependent relationship in the secondary death cascade affecting retinal neurons.

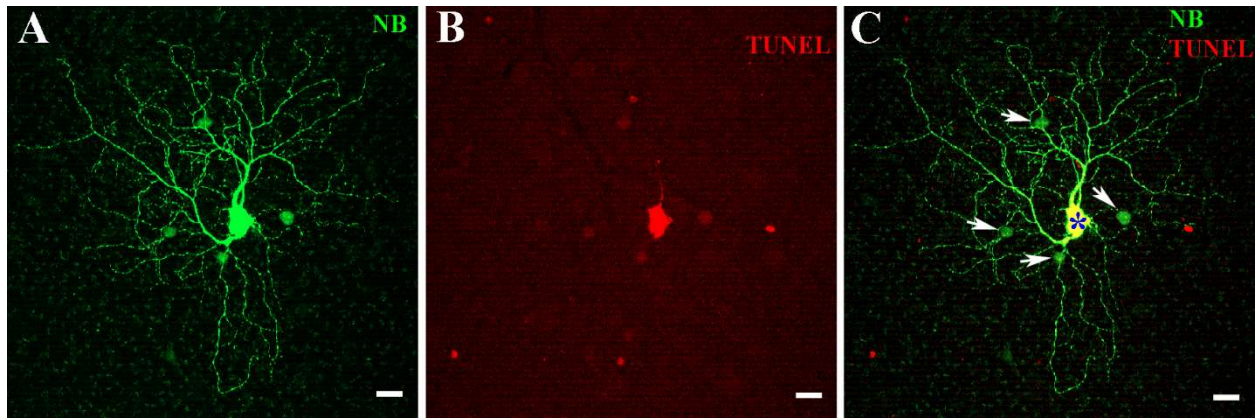


Figure 2.3.2 Gap junction-mediated apoptosis in adjacent neuronal cells following cytochrome C injection.

(A) Representative confocal image of an α RGC along with its coupled neuronal cells in a whole-mount retina. The cell was injected with a mixture of Neurobiotin to visualize gap junction coupling and cytochrome C to induce apoptosis.

(B) Coupled apoptotic neuronal cells were labeled using TUNEL staining.

(C) The overlay of panels A and B demonstrates that apoptosis extends to neighboring neuronal cells (white arrows) coupled to the injected α RGC (asterisk).

Scale bars represent 20 μ m.

2.3.3 Blocking Cx36-related gap junctions may significantly reduce cell death in the far-peripheral and mid-peripheral retina following six-hour corneal alkali burns: analysis in genetic and pharmacological mice

To investigate the potential protective effect of a Cx36-related gap junction antagonist on RGC apoptosis following corneal alkali burns, we first established a six-hour corneal alkali burn model in both WT and Cx36 KO HOMO mice, as this time point maintains relatively high RGC survival rates while allowing effective investigation of secondary cell death mechanisms (**Fig. 2.3.3A**). This allowed us to observe α RGC apoptosis in the far-peripheral, mid-peripheral, and central retinal areas. The primary difference between WT and Cx36 KO HOMO mice was observed in the mid-peripheral retina. As illustrated in **Fig. 2.3.3C**, compared to the far-peripheral (0.90 ± 0.02 , $n = 8$) and mid-peripheral (0.53 ± 0.09 , $n = 8$) retina of 6 hours in the WT group, there was a notable reduction in TUNEL-positive reactions in the far-peripheral area (0.36 ± 0.06 , $n = 8$) and the mid-peripheral area (0.06 ± 0.01 , $n = 8$) of the 6-hour Cx36 KO HOMO mice group. The density of TUNEL/Brn3a double-positive cells in this region was significantly lower in Cx36 KO HOMO mice (the far-peripheral = 917 ± 146 cells/mm² and the mid-peripheral = 159 ± 37 cells/mm²) compared with WT mice (the far-peripheral = 2626 ± 124 cells/mm² and the mid-peripheral = 1889 ± 128 cells/mm²; **Fig. 2.3.3E**). Statistical analysis confirmed a significant decrease in TUNEL-positive reactions for Cx36 KO HOMO mice in this area.

To further investigate the therapeutic potential of Cx36-related gap junction protection, we administered MFA, a known gap junction blocker, via intravitreal injection as our experimental treatment, using Ringer's solution as the control (**Fig. 2.3.3B**). We quantified RGC apoptosis across the far-peripheral, mid-peripheral, and central retinal regions to assess the protective efficacy of MFA treatment (**Fig. 2.3.3D**). MFA treatment demonstrated significant neuroprotective effects that paralleled our genetic knockout findings. A significant decrease in RGC apoptosis was noted in the far-peripheral (0.34 ± 0.03 , $n = 8$) and the mid-peripheral retina following MFA application (0.22 ± 0.02 , $n = 8$). In contrast, without MFA treatment (using Ringer solution as the control), a significantly higher number of TUNEL-positive reactions were observed in the far-peripheral (0.87 ± 0.03 , $n = 8$) and the mid-peripheral retina (0.51 ± 0.03 , $n = 8$). Consistently, the density of TUNEL/Brn3a double-positive cells in this area was significantly lower in MFA-treated mice (the far-peripheral = 903 ± 130 cells/mm² and the mid-peripheral = 688 ± 54 cells/mm²) compared to the Ringer-treated control group (the far-peripheral = 2290 ± 230 cells/mm² and the mid-peripheral = 1633 ± 205 cells/mm²; **Fig. 2.3.3F**).

This substantial reduction in TUNEL-positive reactions confirmed that pharmacological gap junction blockade could effectively replicate the protective phenotype observed in Cx36 knockout mice following a six-hour corneal alkali burn, supporting the therapeutic potential of targeting gap junction-mediated cell death pathways in retinal injury.

To investigate whether alkali could directly affect retinal neurons, we administered an intraocular injection of 1.5 μ l of 0.1 M HEPES buffer into the vitreous cavity to observe the extent of RGC apoptosis. HEPES is used as a buffering agent to maintain pH stability at approximately 7.5. Ringer's solution was used as a control. Six hours after inducing an alkali burn on the cornea, we

observed no significant differences in the far-peripheral, mid-peripheral, and central regions between the HEPES group (far-peripheral = 0.89 ± 0.02 , mid-peripheral = 0.51 ± 0.04 , and the central regions = 0.01 ± 0.01 , $n = 6$) and the Ringer's solution control group (far-peripheral = 0.87 ± 0.03 , mid-peripheral = 0.49 ± 0.02 , and the central regions = 0.01 ± 0.01 , $n = 8$; **Fig. 2.3.3G**).

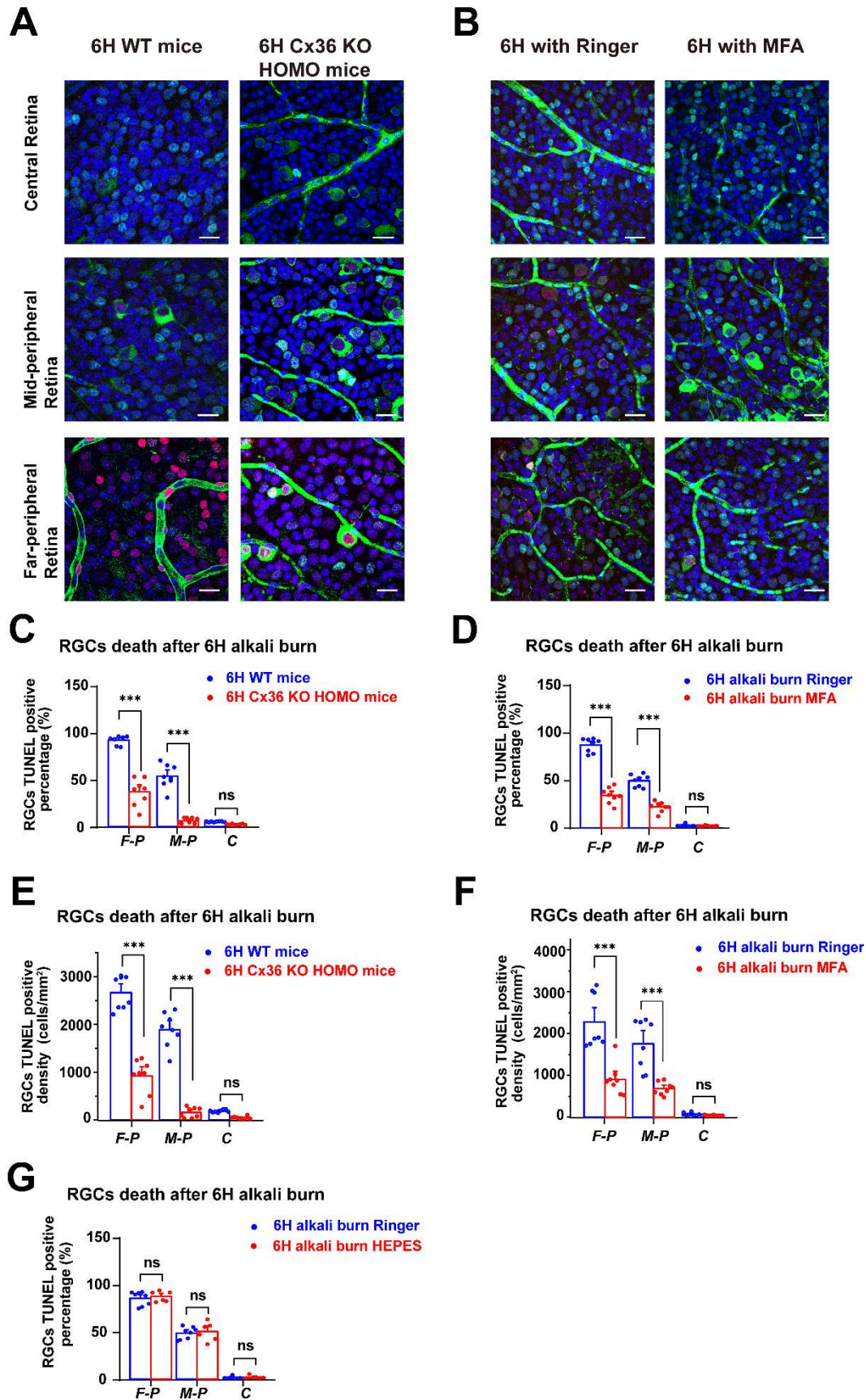


Figure 2.3.3 Retinal damage following six-hour corneal alkali burns.

(A) Representative confocal images of retinal whole-mounts from WT mice and Cx36 KO HOMO mice, 6 hours post-corneal alkali burn. (Green: Brn3a; Red: TUNEL; Blue: DAPI)

(B) Representative confocal images of retinal whole-mounts from WT mice treated with Ringer and MFA after 6 hours post-corneal alkali burns. (Green: Brn3a; Red: TUNEL; Blue: DAPI)

(C) Percentage of TUNEL-positive nuclei in RGCs across three regions in WT and Cx36 KO HOMO mice after 6 hours post-corneal alkali burns. (F-P: far-peripheral, M-P: mid-peripheral, and C: central retinas)

(D) Percentage of TUNEL-positive nuclei in RGCs across three regions in WT mice treated with Ringer and MFA after 6 hours post-corneal alkali burns.

(E) Density of TUNEL-positive nuclei in RGCs across 3 regions in WT and Cx36 KO HOMO mice after 6 hours post-corneal alkali burns.

(F) Density of TUNEL-positive nuclei in RGCs across 3 regions in WT mice treated with Ringer's solution and MFA after 6 hours post-corneal alkali burns.

(G) Percentage of TUNEL-positive nuclei in RGCs across three regions in WT mice treated with intraocular injection of 1.5 μ l of 0.1 M HEPES buffer and Ringer's solution 6 hours post-corneal alkali burns.

Statistical analysis was performed using unpaired t-test. *Scale bars* represent 20 μ m.

2.3.4 No significant morphological changes in ON and OFF α RGCs following six-hour corneal alkali burn

To assess the morphological impact of six-hour corneal alkali burns on ON and OFF α RGCs, we analyzed soma diameter and dendritic field diameter as primary structural parameters (**Fig. 2.3.4A and 2.3.4B**). Soma size measurements demonstrated no statistically significant alterations in either cell type following alkali burn treatment. WT group showed ON α RGCs at $21.24 \pm 0.59 \mu\text{m}$ and OFF α RGCs at $19.87 \pm 0.26 \mu\text{m}$ ($n = 5$), while post-burn values at 6 hours post-corneal alkali burn were $19.87 \pm 0.26 \mu\text{m}$ for ON α RGCs and $19.56 \pm 0.14 \mu\text{m}$ for OFF α RGCs ($n = 5$) (**Fig. 2.3.4C and 2.3.4D**). Dendritic field diameter analysis yielded comparable results, with no significant morphological changes detected. Dendritic field measurements recorded $348.80 \pm 26.70 \mu\text{m}$ for ON α RGCs and $269.30 \pm 18.33 \mu\text{m}$ for OFF α RGCs ($n = 5$) in the WT group, compared to 6 hours post-corneal alkali burn group of $313.10 \pm 31.75 \mu\text{m}$ for ON α RGCs and $268.50 \pm 17.84 \mu\text{m}$ for OFF α RGCs ($n = 6$) (**Fig. 2.3.4E and 2.3.4F**).

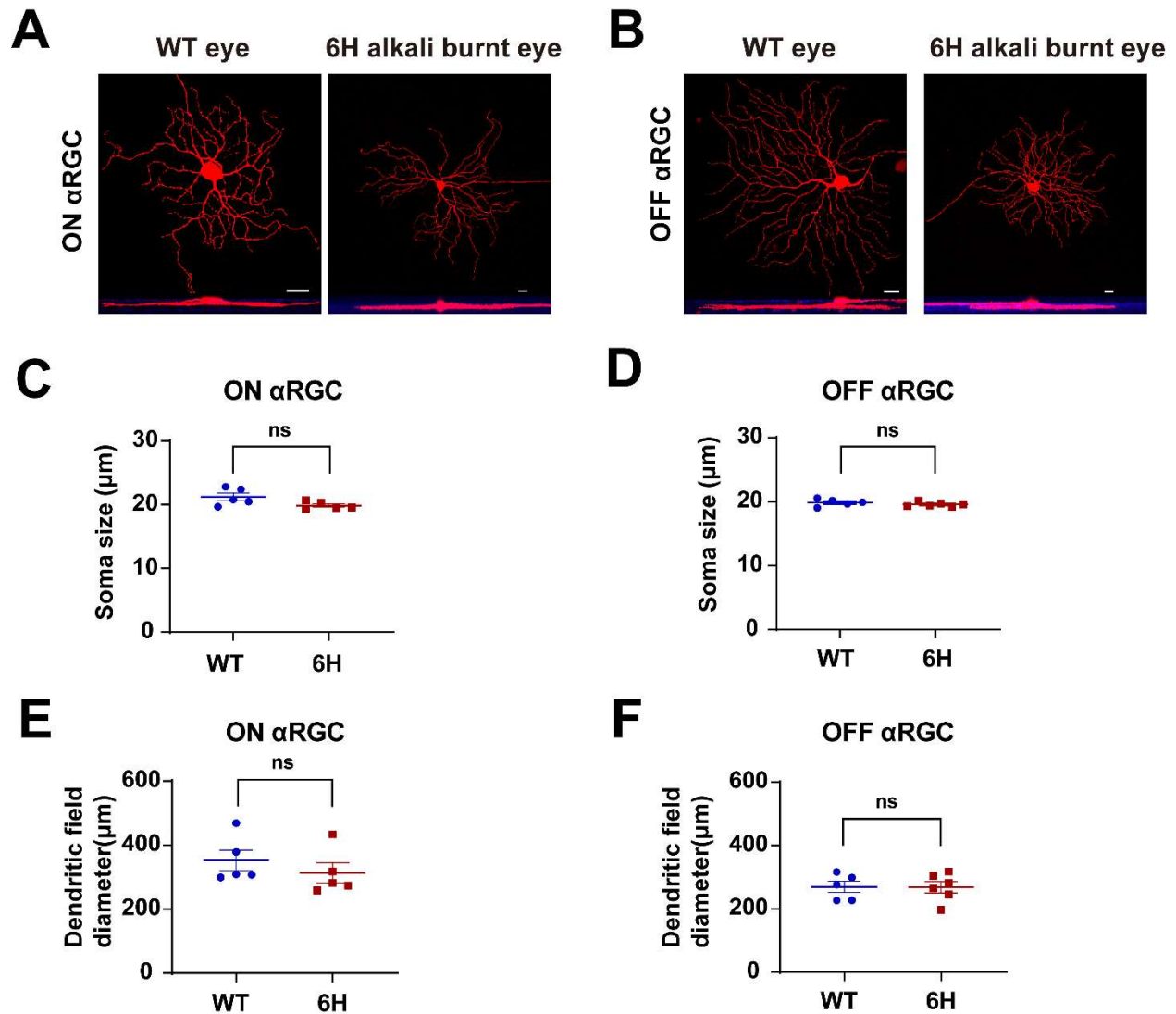


Figure 2.3.4 ON and OFF α RGCs in the retinas of WT mice before and after six-hour corneal alkali burns.

(A, B) Representative images of ON and OFF α RGCs in the mouse retina. ON and OFF α RGCs were labelled with anti-ChAT antibody (blue).

(C, D) The soma diameter size of ON and OFF α RGCs showed no statistical difference between the WT mice retinas and those subjected to six-hour corneal alkali burns.

(E, F) Similarly, the dendritic field diameter of ON and OFF α RGCs showed no statistical difference between the WT mouse retinas and those after six-hour corneal alkali burns.

Scale bars represent 20 μ m.

2.3.5 Corneal alkali burns reduce light-evoked spike responses in ON and OFF transient α RGCs

The light sensitivity of RGCs was evaluated to determine the effects of a six-hour corneal alkali burn. Four distinct α RGC subtypes were identified through their characteristic responses to 525 nm full-field light stimulation: ON transient, ON sustained, OFF transient, and OFF sustained categories (**Fig. 2.3.5A-D**).

Analysis of light-evoked spike response patterns revealed pronounced rightward shifts in the response curves for both ON transient and OFF transient RGCs following corneal alkali burn treatment, as demonstrated in **Fig. 2.3.5E and 2.3.5G**, respectively, when compared to wild-type controls. The ON transient RGCs exhibited a threshold shift from 1.0×10^2 Rh*/rod sec in control conditions to 4.7×10^3 Rh*/rod second post-burn (**Fig. 2.3.5E**), while OFF transient RGCs showed a comparable shift from 5.0×10^2 to 5.0×10^3 Rh*/rod second (**Fig. 2.3.5G**). These threshold elevations indicate compromised light sensitivity following corneal injury. In contrast, sustained α RGC subtypes displayed minimal changes, with ON sustained RGCs showing threshold values from 6.3×10^2 to 1.3×10^3 Rh*/rod second (**Fig. 2.3.5F**) and OFF sustained RGCs remaining relatively stable from 0.5×10^2 to 0.6×10^2 Rh*/rod second (**Fig. 2.3.5H**).

Quantitative analysis confirmed that spike response thresholds for transient α RGC subtypes in six-hour alkali-burned retinas (ON transient α RGCs: 3.92 ± 0.13 , $n = 5$; OFF transient α RGCs: 3.71 ± 0.29 , $n = 5$) were significantly elevated compared to wild-type controls (ON transient α RGCs: 2.79 ± 0.21 , $n = 6$; OFF transient α RGCs: 2.38 ± 0.27 , $n = 5$), representing an increase with statistical significance. These results are summarized graphically in Fig. 2.3.5I.

Notably, sustained α RGC subtypes showed no significant threshold differences between wild-type conditions (ON sustained α RGCs: 2.34 ± 0.29 , $n = 4$; OFF sustained α RGCs: 2.50 ± 0.39 , $n = 5$) and post-burn measurements (ON sustained: 3.16 ± 0.41 , $n = 4$; OFF sustained: 2.63 ± 0.36 , $n = 5$), as visualized in Fig. 2.3.5F, 2.3.5H, and 2.3.5I. This differential response pattern between transient and sustained α RGC populations suggests subtype-specific vulnerability to corneal alkali burn-induced retinal dysfunction.

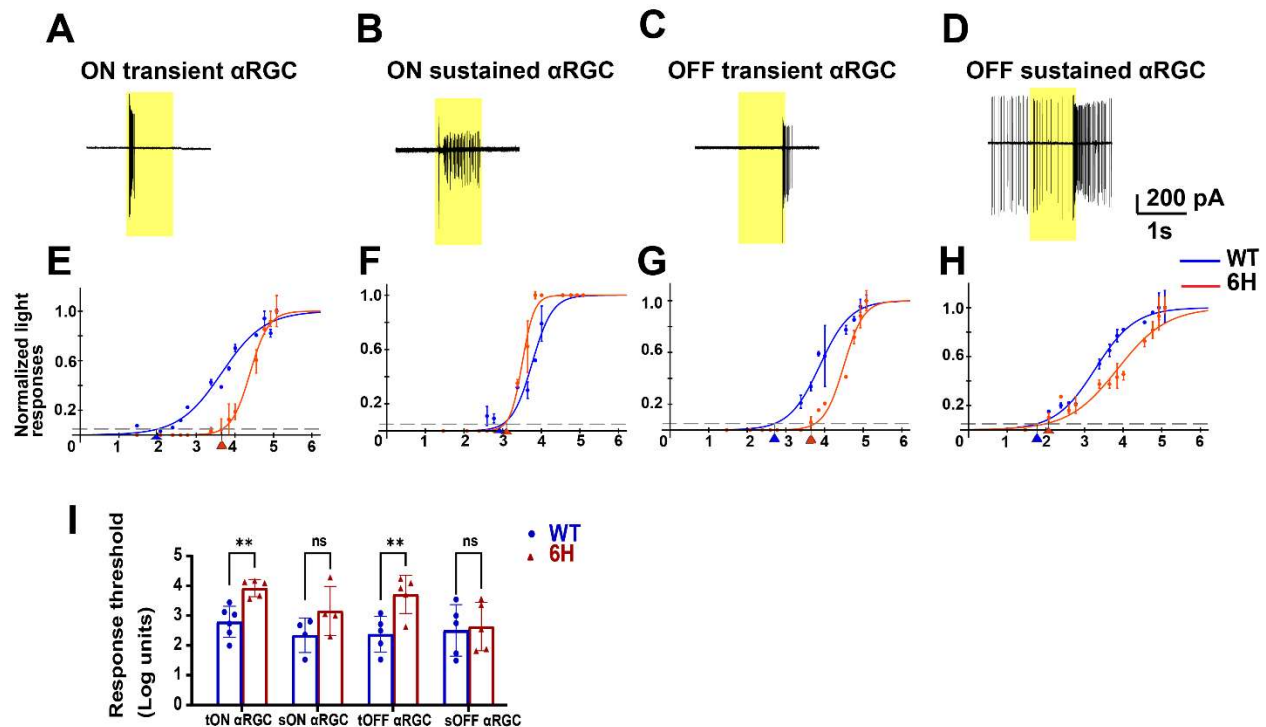


Figure 2.3.5 Light responses of α RGCs in WT and six-hour Alkali Burn Groups.

(A-D) Example spike responses of ON transient, ON sustained, OFF transient, and OFF sustained α RGCs to full-field 525 nm light stimuli.

(E-H) Fitted normalized response-intensity curves for the four α RGC subtypes in the WT group (blue) and the six-hour alkali burn group (red).

(I) Light response thresholds for the four α RGC types in wild-type and six-hour alkali burn mice. Average thresholds are defined as the light intensity eliciting 5% of the maximum spike response.

2.3.6 Substantial reduction in light-evoked EPSCs/IPSCs in ON and OFF α RGCs after six-hour corneal alkali burn

Using whole-cell recording techniques, we aimed to determine whether corneal alkali burns affect the strength of light-evoked excitatory postsynaptic currents (EPSCs) at holding potentials of -68 mV and inhibitory postsynaptic currents (IPSCs) at +20 mV in α RGCs.

ON α RGCs in wild-type mice demonstrated robust responses with large, sustained inward EPSCs (626.50 ± 24.7 pA) and sustained outward IPSCs (465.00 ± 16.49 pA). Following a six-hour corneal alkali burn, ON α RGCs exhibited dramatically reduced synaptic strength, displaying smaller, transient inward EPSCs (129.20 ± 8.37 pA) and transient outward IPSCs (195.10 ± 6.20 pA) (**Fig. 2.3.6A**). Quantitative analysis revealed that average peak amplitudes for ON α RGCs were significantly reduced in the alkali burn group (EPSCs: 91.84 ± 13.18 pA, $n = 9$; IPSCs: 100.30 ± 19.44 pA, $n = 7$) compared to WT group (EPSCs: 429.2 ± 66.26 pA, $n = 6$; IPSCs: 359.80 ± 61.62 pA, $n = 6$) (**Fig. 2.3.6C**, Welch's t-test).

OFF α RGCs displayed similar reductions in strength following corneal injury. Representative recordings in EPSCs showed that OFF α RGCs shifted from large, sustained inward currents (558.90 ± 38.59 pA) to smaller, transient currents (93.70 ± 4.00 pA) post-burn, with outward IPSC recordings showing a comparable decline from 318.8 ± 25.6 pA to 107.1 ± 0.90 pA (**Fig. 2.3.6B**). Statistical comparison revealed that OFF α RGCs in the burn group (EPSCs: 85.70 ± 3.55 pA, $n = 5$; IPSCs: 73.20 ± 12.87 pA, $n = 5$) exhibited significantly reduced average peak amplitudes compared to WT group (EPSCs: 379.30 ± 84.50 pA, $n = 7$; IPSCs: 317.00 ± 32.53 pA, $n = 9$) (**Fig. 2.3.6D**, Welch's t-test).

Charge transfer analysis during one-second light stimulation revealed significant impairment in ON α RGCs following alkali burn treatment. Both EPSC and IPSC charge transfer were substantially diminished in the burn group (EPSC: 98.52 ± 14.16 pC, $n = 9$; IPSC: 87.22 ± 10.57 pC, $n = 9$) compared to WT group (EPSC: 223.90 ± 42.69 pC, $n = 6$; IPSC: 173.50 ± 24.60 pC, $n = 6$) (**Fig. 2.3.6E**, unpaired t-test for IPSC and Welch's t test for EPSC).

OFF α RGCs demonstrated similarly compromised charge transfer capacity post-injury. The differences in charge transfer between groups were statistically significant for both EPSCs (WT: 147.60 ± 10.83 pC, $n = 5$; 6H: 47.77 ± 3.07 pC, $n = 5$) and IPSCs (WT: 248.70 ± 57.63 pC, $n = 6$; 6H: 3.63 ± 6.08 pC, $n = 5$) (**Fig. 2.3.6F**, Welch's t-test).

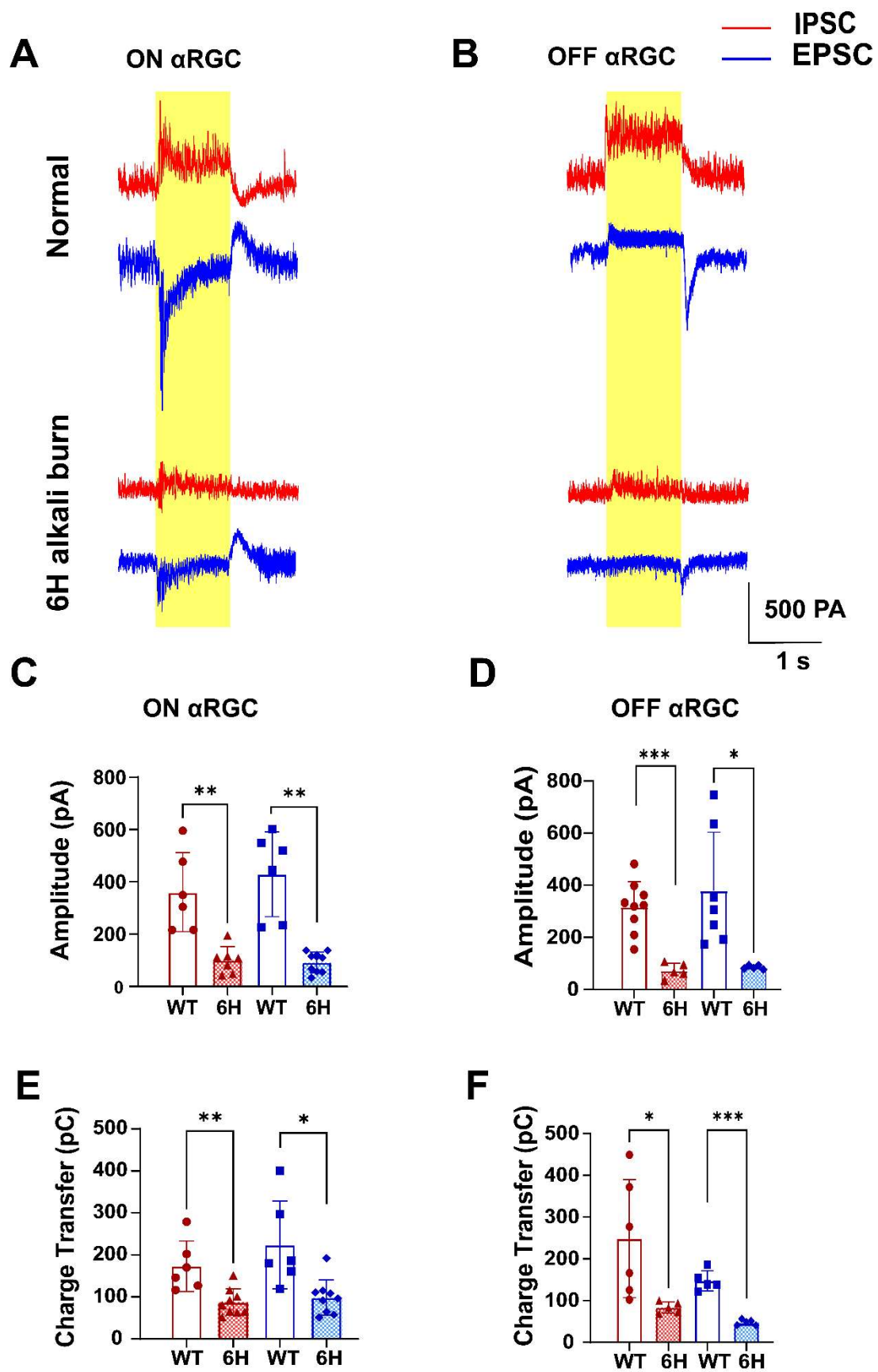


Figure 2.3.6 Light-evoked EPSCs and IPSCs in ON and OFF α RGCs of WT and six-hour alkali burn groups.

EPSCs and IPSCs were recorded at holding potentials of -68 mV and +20 mV, respectively, with EPSCs shown in blue and IPSCs in red.

(A, B) Images of light-evoked EPSCs and IPSCs in ON and OFF α RGCs from WT and six-hour alkali burn mice.

(C) Average peak amplitudes of light-evoked EPSCs and IPSCs in ON α RGCs.

(D) Average peak amplitudes of light-evoked EPSCs and IPSCs in OFF α RGCs.

(E) Average total charge transferred by light-evoked EPSCs and IPSCs in ON α RGCs.

(F) Average total charge transferred by light-evoked EPSCs and IPSCs in OFF α RGCs.

2.3.7 MFA treatment reduces light sensitivity loss of ON and OFF transient α RGC following a six-hour alkali burn

We explored whether the gap junction antagonist MFA could mitigate the impaired light response threshold observed in transient α RGCs after corneal alkali burn injury. Following burn induction, mice were allocated into two experimental cohorts: one group received intravitreal MFA injections as treatment, while the control group received intravitreal Ringer's solution injections for comparative analysis.

Representative recordings demonstrate that ON transient α RGCs in the MFA-treated group (4.10×10^2 Rh*/rod second) exhibited enhanced light-evoked sensitivity compared to the Ringer's

solution control group (9.12×10^3 Rh*/rod second), as shown in **Fig. 2.3.7A**. This improved responsiveness was consistently observed across multiple full-field light sensitivity levels (2.4×10^3 , 1×10^4 and 8.4×10^4 Rh*/rod second). Light response curve revealed a subtle leftward shift in ON transient RGC, with threshold values shifting from 1.7×10^3 Rh/rod second in the Ringer's solution group to 8.5×10^2 Rh*/rod second following MFA treatment (**Fig 2.3.7C**). However, statistical analysis showed no significant difference in light response thresholds between the MFA group (3.23 ± 0.61 , mean $\pm$ SEM, $n = 4$) and the Ringer's solution group (3.92 ± 0.24 , $n = 4$) for ON transient α RGCs (**Fig. 2.3.7E**).

OFF transient α RGCs displayed similar patterns of MFA-induced enhancement. As illustrated in **Fig. 2.3.7B**, OFF transient α RGCs in the MFA-treated group (1.70×10^3 Rh*/rod second) demonstrated improved light sensitivity compared to controls receiving Ringer's solution (1.62×10^3 Rh*/rod second). The light response curves for OFF transient RGCs showed a corresponding leftward shift with MFA treatment, with thresholds decreasing from 5.00×10^3 Rh*/rod second in the Ringer's solution group to 3.98×10^3 Rh*/rod sec after MFA application (**Fig. 2.3.7D**). Despite these favorable trends toward earlier spike initiation and leftward curve shifts, statistical comparison revealed no significant difference in light response thresholds between the MFA group (3.32 ± 0.17 , $n = 10$) and the Ringer's solution group (3.79 ± 0.22 , $n = 6$) for OFF transient α RGCs (**Fig. 2.3.7F**). These findings suggest that while MFA treatment may provide subtle improvements in light responsiveness, it does not achieve statistically significant restoration of light response thresholds under the current experimental parameters.

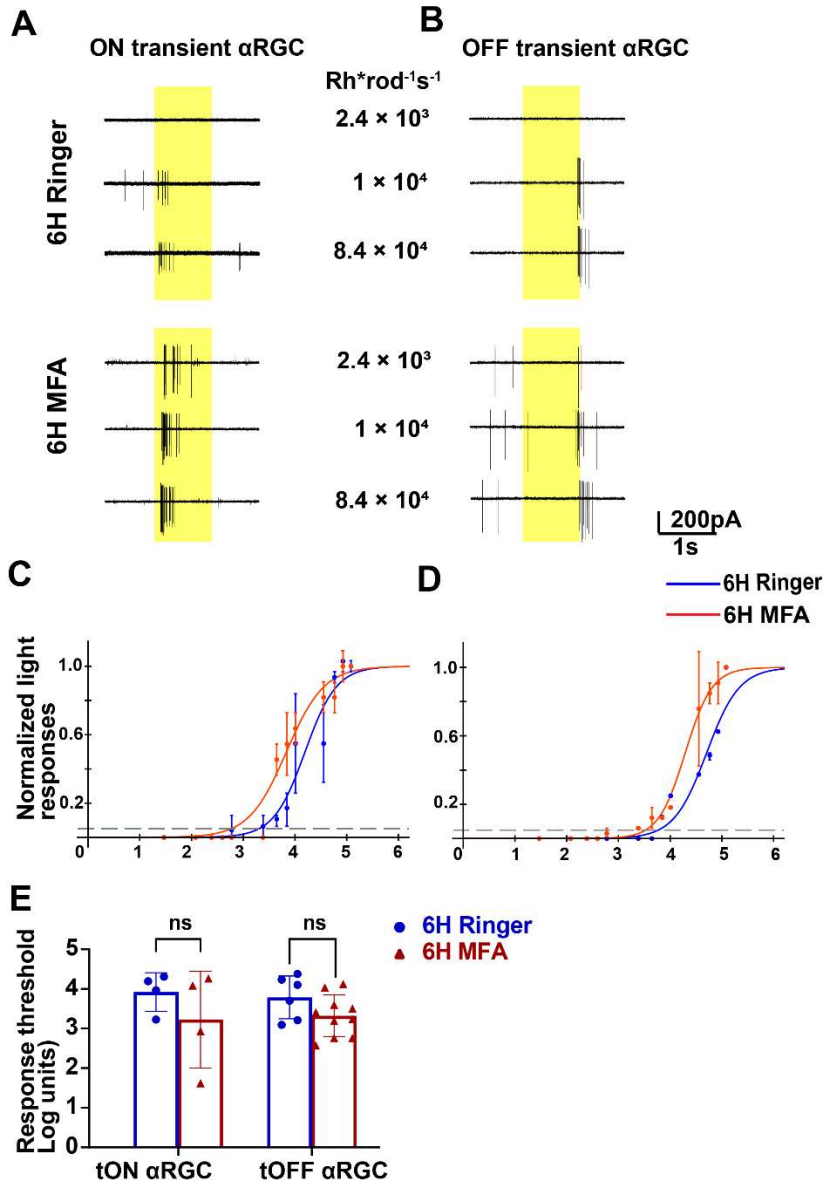


Figure 2.3.7 Light responses of ON and OFF transient α RGCs in six-hour alkali burn of Ringer and MFA-treated groups.

(A, B) Example spike responses of ON and OFF transient α RGCs to full-field 525 nm light stimuli at varying intensities.

(C, D) Normalized light response-intensity curves for the two transient α RGC subtypes in the six-hour alkali burn in Ringer (blue) and the MFA-treated (red) groups.

(I) Light response thresholds for the two α RGC subtypes in the six-hour alkali burn Ringer and MFA-treated groups. Average thresholds are defined as the light intensity eliciting 5% of the maximum spike response.

2.3.8 The effect of MFA on EPSCs in ON α RGCs

We examined whether MFA administration could prevent the diminished input signals to RGCs by recording light-evoked EPSCs and IPSCs in two subtypes of α RGCs after corneal alkali burn injury. Experimental animals received intravitreal injections of MFA, while control animals received equivalent volumes of Ringer's solution via the same injection route.

Representative electrophysiological recordings from ON α RGCs are presented in **Fig. 2.3.8A**, displaying EPSCs measured at -68 mV and IPSCs measured at +20 mV following 1-second light exposure at 525 nm wavelength for each experimental group. Following MFA treatment, ON α RGCs demonstrated significant alterations in their electrophysiological responses to full-field light stimulation. Specifically, EPSCs showed enhanced inward current amplitudes, with values rising from 136.70 ± 8.30 pA under control Ringer's solution conditions to 149.50 ± 2.67 pA in the presence of MFA. Similarly, IPSCs displayed moderately increased outward current magnitudes, with amplitudes increasing from 70.80 ± 2.48 pA in Ringer's solution to 101.30 ± 0.75 pA following MFA application (**Fig. 2.3.8A**).

MFA treatment produced significant modifications in the EPSCs of ON α RGCs, demonstrating a marked enhancement in peak amplitude during full-field light stimulation (Ringer: 109.70 ± 12.41 pA, $n = 9$; MFA: 138.00 ± 10.40 pA, $n = 5$; unpaired t-test) (**Fig. 2.3.8C**). Conversely, analysis of EPSC charge transfer revealed no statistically significant difference between treatment conditions

(**Fig. 2.3.8E** Ringer: 95.01 ± 10.97 pC, $n = 7$; MFA: 100.00 ± 11.19 pC, $n = 5$; unpaired t-test), suggesting that the overall synaptic charge remains stable despite amplitude changes. For IPSCs, MFA application resulted in elevated mean peak amplitudes compared to control conditions, though statistical comparison revealed no significant difference between the MFA group (87.74 ± 18.79 pA, $n = 5$) and the Ringer group (67.48 ± 6.01 pA, $n = 8$) (**Fig. 2.3.8C**, Welch's t-test). Consistent with the excitatory findings, IPSC charge transfer analysis demonstrated no significant difference between experimental groups (Ringer: 86.91 ± 11.29 pC, $n = 8$; MFA: 90.49 ± 9.75 pC, $n = 5$) (**Fig. 2.3.8E**, unpaired t-test).

To investigate MFA's impact on OFF α RGC input signals, we conducted recordings of both EPSCs and IPSCs (**Fig. 2.3.8B**). The measured EPSC amplitudes showed minimal variation between conditions, registering 128.2 ± 6.10 pA in Ringer solution compared to 128.1 ± 2.89 pA following MFA treatment, while IPSC amplitudes similarly remained stable at 102.8 ± 7.75 pA in Ringer versus 101.3 ± 2.96 pA with MFA (**Fig. 2.3.8B**). Statistical analysis of peak amplitudes revealed no significant differences between treatment groups for either current type (Ringer, EPSCs: 109.1 ± 7.79 pA, $n = 5$; MFA EPSCs: 108.4 ± 6.39 pA, $n = 8$; unpaired t-test; Ringer, IPSCs: 81.98 ± 18.49 pA, $n = 5$; MFA IPSCs: 78.70 ± 10.42 pA, $n = 6$; unpaired t-test) (**Fig 2.3.8D**). Charge transfer measurements likewise demonstrated no statistically significant alterations following MFA administration (Ringer, EPSCs: 75.75 ± 5.63 pC, $n = 5$; MFA, EPSCs: 67.31 ± 1.39 pC, $n = 7$; Welch's t-test; Ringer, IPSC: 68.45 ± 9.96 pC, $n = 5$; MFA, IPSC: 90.10 ± 6.87 pC, $n = 5$; unpaired t-test) (**Fig. 2.3.8F**).

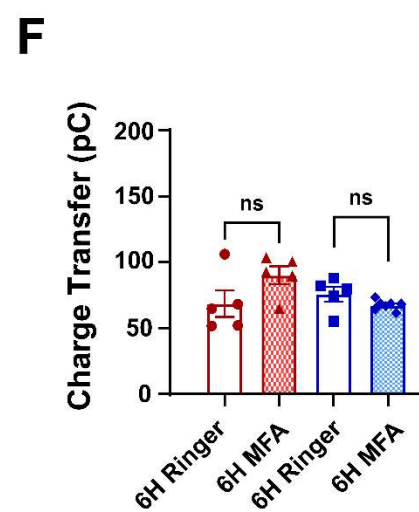
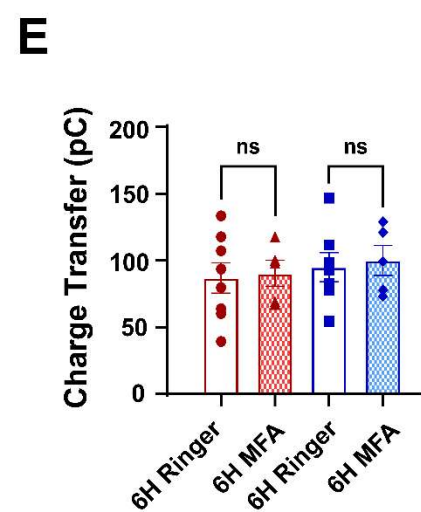
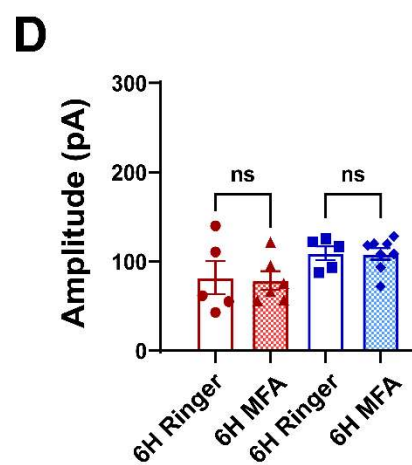
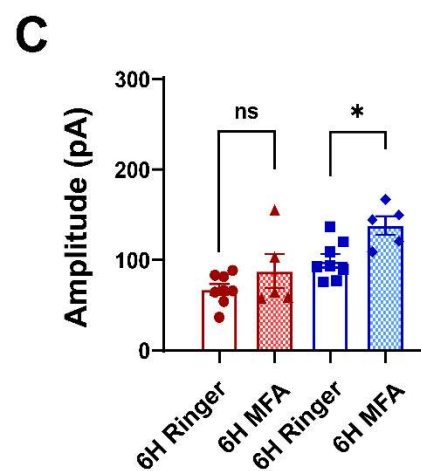
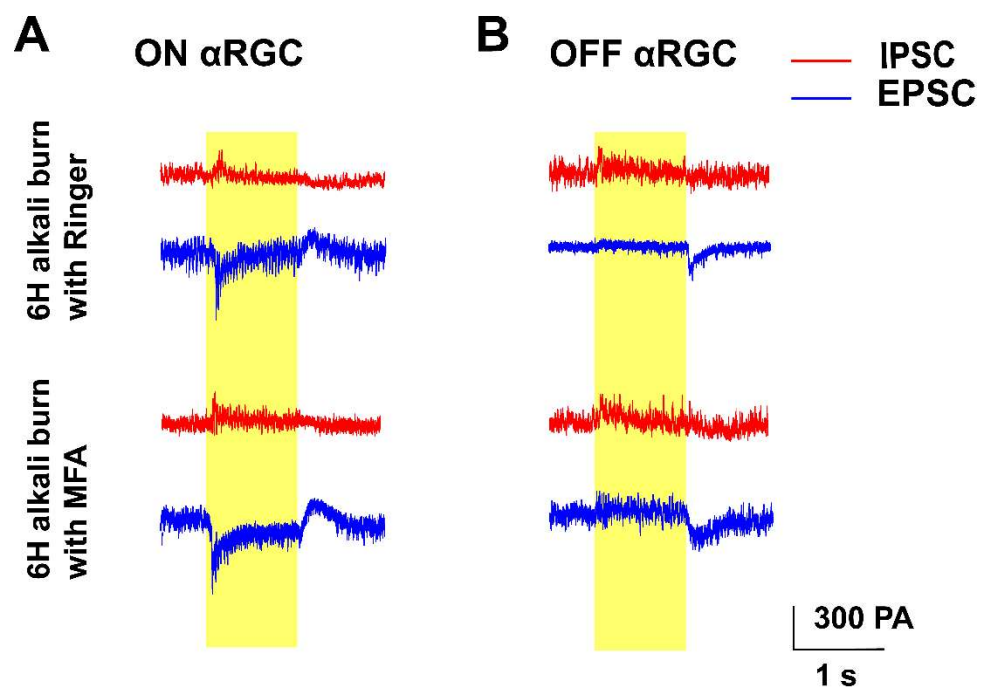


Figure 2.3.8 Light-evoked EPSCs and IPSCs in ON and OFF α RGCs of Wild-type control and six-hour alkali burn groups.

EPSCs and IPSCs were recorded at holding potentials of -68 mV and +20 mV, respectively, with EPSCs shown in blue and IPSCs in red.

(A, B) Images of light-evoked EPSCs and IPSCs in ON and OFF α RGCs from wild-type and six-hour alkali burn mice.

(C) Average peak amplitudes of light-evoked EPSCs and IPSCs in ON α RGCs.

(D) Average peak amplitudes of light-evoked EPSCs and IPSCs in OFF α RGCs.

(E) Average total charge transferred by light-evoked EPSCs and IPSCs in ON α RGCs.

(F) Average total charge transferred by light-evoked EPSCs and IPSCs in OFF α RGCs.

2.3.9 Microglial activation following corneal alkali burns

To examine whether microglia are activated following corneal alkali burn, we employed Anti Iba1 immunostaining to assess alterations in microglial populations and evaluate changes in microglial cell numbers.

First, we observed a significant increase in microglial cell numbers in the retinas of wild-type mice at 6 hours post-alkali burn injury (**Fig. 2.3.9A**). Upon further regional analysis, we found that microglial density increased from 43 ± 2 to 96 ± 4 cells in the far-peripheral retina, from 56 ± 1 to

97 ± 2 cells in the mid-peripheral retina, and from 65 ± 2 to 105 ± 5 cells in the central retina (**Fig. 2.3.9C**; $n = 4$; unpaired t-test).

Next, we investigated the microglial response in Cx36 KO mouse retinas at 6 hours following corneal alkali burn injury (**Fig. 2.3.9B**). Similar to wild-type mice, Cx36 KO mice demonstrated a marked elevation in retinal microglial populations at 6 hours post-injury. Quantitative regional analysis showed that microglial density increased from 44 ± 4 to 80 ± 4 cells in the far-peripheral retina, from 51 ± 3 to 92 ± 5 cells in the mid-peripheral retina, and from 53 ± 5 to 99 ± 5 cells in the central retina (**Fig. 2.3.9D**; $n = 4$; unpaired t-test).

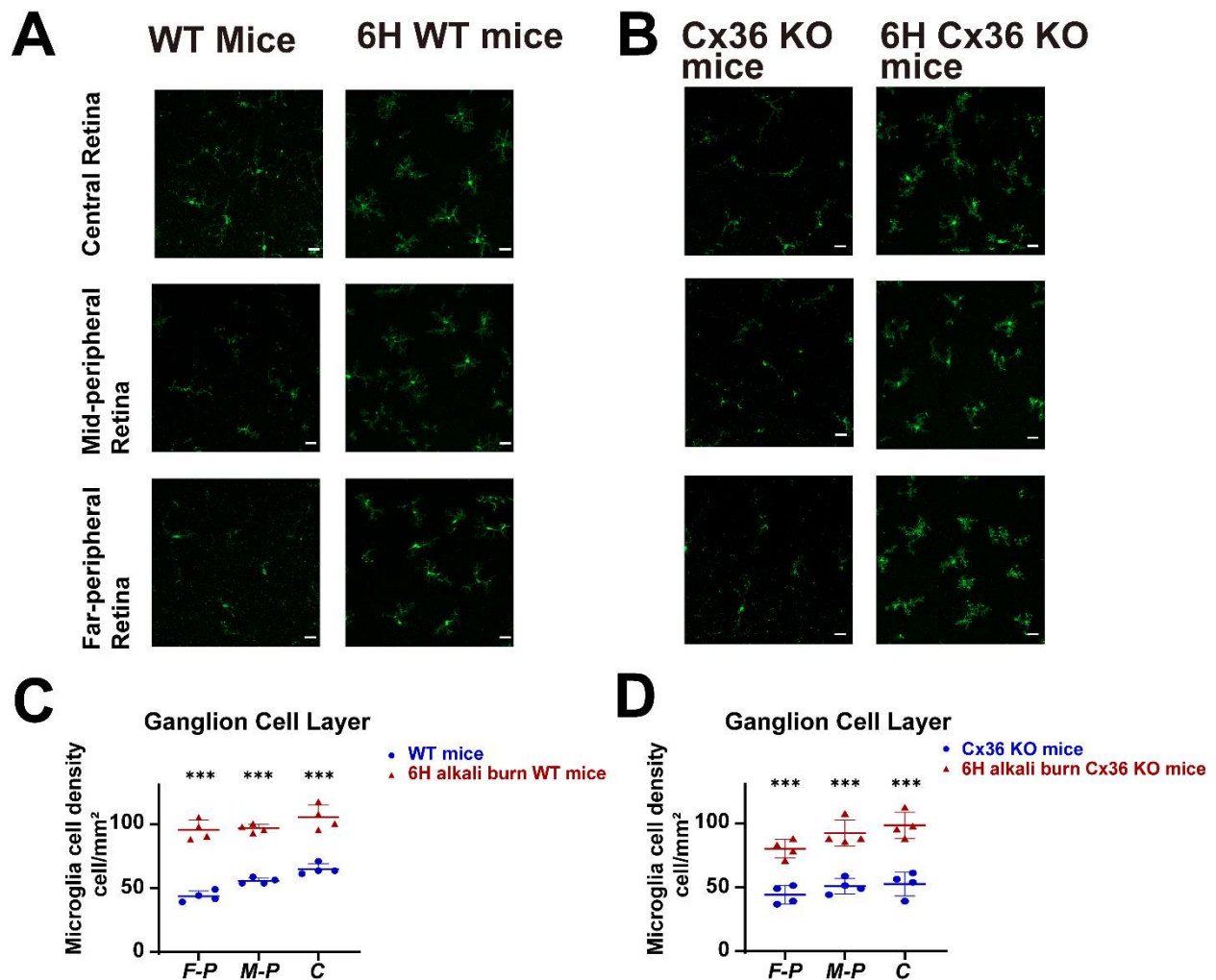


Figure 2.3.9 Microglial changes following a six-hour corneal alkali burn.

(A) Confocal images illustrating the far-peripheral (F-P), mid-peripheral (M-P), and central (C) regions of the retinas in WT and six hours after corneal alkali burn. Microglia were visualized using anti-Iba1 immunolabeling (green).

(B) Confocal images of the far-peripheral, mid-peripheral, and central retinas in Cx36 KO HOMO mice, both untreated and six hours after alkali burn. Microglia were visualized using anti-Iba1 immunolabeling (green).

(C) Quantitative analysis of microglial density in WT mice, comparing untreated and 6-hour alkali burn conditions.

(D) Quantitative analysis of microglial density in Cx36 KO HOMO mice, comparing untreated and 6-hour alkali burn conditions.

Scale bars represent 20 μm .

2.4 Discussion

2.4.1 Apoptosis of RGCs in corneal alkali burn: A six-hour post-burn model

Corneal alkali burns can induce apoptosis in various subtypes of α RGCs, starting in the far-peripheral retina and gradually spreading to the mid-peripheral and central regions. A six-hour post-injury timeframe was chosen to examine the effects of alkali-induced corneal injury on retinal neurons, as it demonstrated a significant temporal and spatial progression of TUNEL-positive RGCs. Extending the observation period to 12 hours results in excessive apoptosis, complicating the study of biophysical properties.

The reduction in light sensitivity, particularly in transient α RGCs, may have important implications for visual processing and could contribute to visual disturbances following corneal injuries. These findings indicate that within the acute six-hour period post-burn, both ON and OFF α RGCs experience significant impairment in light-evoked synaptic inhibition and excitation. This comprehensive alteration in retinal circuitry suggests a rapid and widespread impact of corneal injury on visual processing at the retinal level.

The observed changes in excitatory and inhibitory currents may contribute to the altered light sensitivity and visual disturbances associated with corneal alkali burns. Further research is necessary to elucidate the underlying mechanisms of these rapid functional changes and to explore potential therapeutic interventions that could mitigate the impact of corneal injuries on retinal function.

2.4.2 Alkali did not play a key role in the apoptosis of RGCs following corneal alkali burns

The mechanism of apoptosis in RGCs following corneal alkali burns involves a complex interaction of caspase activation and various apoptotic pathways. RGCs are particularly vulnerable to damage, leading to their programmed cell death through caspase-dependent mechanisms (Choudhury et al., 2015).

Alkali burns may induce RGC apoptosis through inflammatory responses and oxidative stress. These burns could cause an increase in TNF- α , which triggered RGC apoptosis via inflammatory pathways. This effect is intensified by the infiltration of peripheral monocytes and the activation of microglia, which releases more TNF- α , further damaging the retina (Paschalis et al., 2018). Additionally, the activation of NADPH oxidases could boost the production of reactive oxygen species, intensifying oxidative stress and inflammation, both of which are crucial in RGC apoptosis (Gu et al., 2016).

To assess whether alkali base directly impacts the retina, we injected HEPES buffer into the vitreous cavity as a pH buffer to observe if it affected RGC apoptosis. The Ringer solution served as vehicle control. Six hours after the corneal alkali burn, there was no significant difference in RGC apoptosis between the HEPES and Ringer control groups. This suggested that alkali may not be a primary factor in RGC death following corneal alkali burns. The vitreous body is substantial and effectively buffers sudden pH changes, providing protection to the eye.

2.4.3 Microglial activation and RGCs' apoptosis following corneal alkali burns

Apoptosis of RGCs following a corneal alkali burn may result from multiple mechanisms. To investigate potential immune-mediated contributions to RGC apoptosis, we examined the activation and quantitative changes in microglial cells post-burn (**Fig. 2.3.9**). Microglia, the primary immune cells of the central nervous system, exhibited rapid and robust activation, signalling a significant immune response within the retina. This response may contribute to RGC apoptosis through the release of pro-inflammatory cytokines, reactive oxygen species, or other neurotoxic factors.

The activation of microglia supports the notion that inflammatory responses, primarily mediated by retinal cytokines, play a crucial role in the pathophysiology of ocular alkali burns (Miyamoto et al., 1998; Paschalis et al., 2017). This inflammatory component may complicate the pathogenesis of retinal damage following corneal alkali burns. There is a significant increase in microglial cells after an alkali burn, particularly in the far-peripheral area, the mid-peripheral area, and the central area. Similar results were observed in Cx36 KO HOMO mice retinas, with a significant increase in microglial cells in the far-peripheral area, the mid-peripheral area, and the central area. These findings suggest that the increased presence of microglial cells indicates that inflammatory responses play a crucial role in the pathophysiology of ocular alkali burns.

2.4.4 Connexin 36 played a role in acute retinal ganglion cell apoptosis six hours after corneal alkali burn

Our research reveals that the number of apoptotic cells in the retina increases progressively over time following a corneal alkali burn, with apoptosis advancing from the peripheral to the central retina and peaking at 24 hours. These findings were consistent with other animal models of alkali burns, indicating that corneal alkali burns triggered apoptosis of RGCs. A similar progressive pattern of RGC apoptosis was also observed in glaucoma. This similarity may help explain why glaucoma is a frequent complication in patients with ocular alkali burns (Cadetti & Thoreson, 2006).

Electrical synapses, also known as gap junctions, modulate visual signals and function as a regulator of the bidirectional flows of ions and small metabolites between neurons in the nervous system (Bloomfield & Völgyi, 2009). Cx36 is the predominant subunit of gap junctions in the proximal mouse retina. Gap junction-mediated secondary cell death has been implicated in the loss of retinal neurons observed in various degenerative conditions, including retinitis pigmentosa, glaucoma, and ischemia (Das et al., 2008; Ripps, 2002). Cx36, a gap junction protein prevalent in the retinal ganglion cell layer, has been highlighted in recent studies for its role in mediating bystander cell death in the retina. Akopian et al. demonstrated that following cytochrome C injection into a single RGC, neighbouring coupled RGCs exhibited TUNEL-positive reactions, suggesting gap junction-mediated secondary cell death (Akopian et al., 2014). Furthermore, in excitotoxic models, Cx36 KO mice showed a 50% increase in cell survival compared to WT mice (Akopian et al., 2014), indicating Cx36's involvement in regulating secondary cell death.

Six hours post-burn, both Cx36 KO HOMO mice and WT mice treated with MFA exhibited significantly reduced retinal apoptosis compared to controls. These findings underscore the role of Cx36 in mediating secondary cell death following corneal alkali burns. The protective effects observed with both genetic knockout and pharmacological inhibition of Cx36 not only corroborate previous findings but also enhance our understanding of Cx36's involvement in corneal alkaline injuries. We propose that blocking Cx36 offers a neuroprotective effect on the retina after corneal alkali burns.

2.4.5 Protecting RGCs from corneal alkali burn with MFA application

MFA, a gap junction antagonist, has demonstrated potential in protecting RGCs from damage and secondary cell death in experimental glaucoma (Akopian et al., 2014). Already approved by the U.S. Food and Drug Administration for treating joint pain, muscular pain, and arthritis, MFA could play a crucial role in treating ocular alkali burns and preventing sight-threatening conditions if its neuroprotective effects were confirmed. Its existing approval could facilitate a faster regulatory pathway compared to a completely new chemical entity.

The study suggests that MFA may have a protective or restorative effect on RGC apoptosis. While MFA did not significantly affect the light sensitivities of ON and OFF α RGCs, it did induce noticeable changes in both excitatory and inhibitory postsynaptic currents in ON α RGCs, with minimal impact on the synaptic inputs of OFF α RGCs.

These findings could potentially identify new targets for therapeutic intervention in RGC injury and provide evidence for the efficacy of MFA in treating alkali burns, bringing its possible clinical application one step closer.

2.5 Conclusion

Corneal alkali-induced trauma can lead to retinal damage and subsequent vision loss, potentially due to the apoptosis of RGCs. This retinal damage is characterized as progressive and regional retinal ganglion cell death. The number of apoptotic cells in the retina increases with the time of corneal alkali burns. In addition, this appearance presents a trend of expanding from the peripheral to the central part, reaching a peak point at 24 hours. The study suggests that secondary cell death, mediated by the gap junction subunit Cx36, may play a crucial role in this process. Implementing a neuroprotective strategy that inhibits Cx36-mediated secondary cell death could be beneficial in the early stages following corneal alkali injury.

Chapter 3 Pressure-induced Retinal Ganglion

Cell Signal Alterations: Sustained, Acute, and

Direct Mechanical Effects

3.1 Introduction

Glaucoma represents one of the leading causes of irreversible blindness worldwide, affecting over 95 million people globally and characterized by progressive RGC loss and optic nerve degeneration (Jayaram et al., 2023; Quigley & Broman, 2006; Tham et al., 2014). Elevated IOP has been identified as the primary modifiable risk factor in glaucoma development, with clinical evidence demonstrating that IOP reduction can slow or halt disease progression (Heijl et al., 2002; Kass et al., 2002). However, despite decades of research, fundamental questions remain regarding the primary site and mechanisms by which elevated IOP initiates retinal ganglion cell damage.

Traditionally, glaucoma research has focused primarily on the ONH as the initial site of pressure-induced damage. The widely accepted model proposes that elevated IOP causes mechanical deformation of the lamina cribrosa, leading to axonal compression, disrupted axonal transport, and subsequent retrograde RGC death (Quigley & Addicks, 1980; Wallace & O'Brien, 2016). This optic nerve head hypothesis has dominated our understanding of glaucoma pathogenesis for decades and has guided much of the therapeutic development in the field (Downs et al., 2008; Fuchshofer & Tamm, 2012; Hayreh et al., 1999; Schneider & Fuchshofer, 2016).

However, emerging evidence suggests that the retina proper may also be a direct target of elevated IOP. Agar et al. first demonstrated that neuronal apoptosis occurs under physiologically elevated

pressure in vitro, indicating that elevated pressure alone can serve as a trigger for apoptotic pathways (Agar et al., 2000). Guo et al. further reported that RGC apoptosis in glaucoma correlates with IOP-induced extracellular matrix remodeling involving MMP-9 activity, suggesting the retina as the primary site of glaucomatous injury (Guo et al., 2005). Moreover, early synaptic disconnection in RGCs has been observed to precede cell death during ocular hypertension, likely supporting the concept that direct retinal mechanisms contribute significantly to early glaucomatous pathology (Fu et al., 2009). Despite these important observations, direct evidence for retinal vulnerability to elevated pressure remains limited, and the specific mechanisms by which elevated IOP affect RGC function and survival in the retinal environment are poorly understood.

To address these fundamental questions, we employed complementary experimental approaches designed to isolate and characterize the direct effects of elevated pressure on retinal ganglion cells. We utilized both acute and sustained models of ocular hypertension to examine pressure-induced retinal changes and how different temporal patterns of pressure exposure affect RGC structure and function. To create an acute ocular hypertension (AOH) model, a homemade micropipette attached to a saline container was placed in the anterior chamber of WT mice and maintained for 50 minutes, with container height calibrated to induce an intraocular pressure of 75 mmHg. To establish a sustained ocular hypertension (SOH) model, 2 μ l silicone oil was injected into the anterior chamber of WT mice. Additionally, we developed two direct mechanical pressure methods to isolate immediate effects on RGC physiology: pressure controller-based application and direct micropipette pressure application. Through comprehensive assessment of behavioral performance, electrophysiological function, and cellular survival, we sought to determine the relationship

between pressure-induced functional impairment and structural damage, and to elucidate the mechanisms by which mechanical pressure compromises retinal information processing.

3.2 Methodology

3.2.1 Animal preparation

All animals were maintained in a 12-h light/12-h dark cycle in the Centralised Animal Facilities (CAF) in the Hong Kong Polytechnic University. To ensure consistency in experiment, all mouse enucleation procedures were conducted around 10 a.m. after overnight dark adaptation, then retina tissues were collected immediately following enucleation.

Adult C57BL/6J wild-type (WT) mice (RRID: IMSR_JAX:000664), Ai32 mice (RRID: IMSR_JAX:012569), KCNG4^{cre} mice (RRID: IMSR_JAX:029414), and the KCNG-YFP mice of either sex (postnatal days 6–8 weeks old), weighing 15–25 g, were used in the research.

KCNG-YFP mice were generated by crossing Ai32 mice and KCNG4^{cre} mice. In these mice, α RGCs are YFP labeled and were used for injection experiments. KCNG4^{cre} mice carry Cre recombinase inserted at the locus responsible for encoding the potassium channel modulator. In Ai32 mice, the expression of a channelrhodopsin-2/EYFP fusion protein is triggered by the presence of Cre recombinase. Homozygous KCNG4^{cre} mice were identified through genotyping and used for breeding purposes.

3.2.1.1 Mouse model of AOH

Mice were deeply anesthetized via intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) mixture. A drop of lidocaine (20 mg/ml) applied to the surface of the eyeballs served as a local anesthetic for the eye. One drop of 1% tropicamide ophthalmic solution (Mydracyl, Alcon) was used to dilate the mouse pupil. After 5 minutes, when the pupil was fully dilated, the experiment proceeded, preventing damage to the iris and other structures from the needle tip during subsequent experimental procedures.

The mice were placed in a lateral recumbent position on the pad, with the eye to be operated on positioned as vertically upward as possible. Typically, the right eye was operated on.

Sharp micropipettes were prepared in advance: borosilicate glass capillaries were first stretched into fine tips using a puller machine according to a pre-set program, then refined using a micro grinder with water-grinding principles to achieve tips with the required length and diameter. The container with sterile irrigation solution (balanced salt solution, pH = 7.5) was suspended at a height of 1m above the mouse cornea, equivalent to 75 mmHg. Disposable infusion tubing was used to connect the water container to the sharp micropipette. After the connection, the entire setup was tested beforehand to adjust the solution flow rate and ensure there was no blockage in the micropipette.

The mouse was placed under a 10x microscope, ensuring the cornea was completely within the field of view. The sharp micropipette was slowly inserted into the anterior chamber from a position away from the center of the cornea, avoiding contact with the lens, iris, and other ocular structures. The micropipette was then fixed in position and kept stationary in the mouse's anterior chamber for 50 minutes. During this period, the mouse was observed to prevent the movement of the micropipette. After 50 minutes, the micropipette was withdrawn from the mouse's anterior

chamber, and the mouse was placed on a 37 °C heating pad until it recovered. Mice that developed corneal opacity, neovascularization, or other corneal diseases were excluded from the successful experimental subjects.



Figure 3.2.1.1 Illustration of a sharp micropipette being inserted into the anterior chamber.

3.2.1.2 Mouse model of SOH

The mice underwent deep anesthesia by an intraperitoneal injection of the mixture of ketamine (80 mg/kg) /xylazine (10 mg/kg). The mice were then placed in a lateral position on a surgery platform, and the microscope was adjusted to ensure optimal visualization, with the mouse eye centered and fully visible within the microscope's field of view.

Prior to injection, topical anesthesia was administered by applying one drop of lidocaine (20 mg/ml) to the corneal surface to alleviate the mice's pain and discomfort. A 30-G needle was carefully inserted through the corneal layers at the superotemporal quadrant near the ocular limbus, creating a tunnel to access the anterior chamber while avoiding damage to the lens and iris. This position also serves to prevent silicone oil leakage during subsequent operational steps.

Approximately 2 μ l of silicone oil (1,000 mPa·s, Silikon, Alcon Laboratories, Fort Worth, Texas) was pre-loaded into a homemade borosilicate glass capillary. Preparing the tip of a homemade borosilicate glass capillary to the required specifications. The tip of the micropipette was carefully inserted through the established tunnel, and the silicone oil was slowly injected into the ocular anterior chamber using a homemade injector within 1 minute. The progressive expansion of the oil droplet could be observed until it nearly covered the entire iris surface.

The homemade micropipette was maintained in position for 30 seconds before being withdrawn. To minimize the leakage of injected silicon oil, the eyelid was positioned to cover the incision. The contralateral control eyes were punctured in the anterior chamber using a 30-G needle. During the whole procedure, artificial tears were applied to keep the cornea moist. After the experimental procedures, the mice were placed on a 37 °C heating pad until they fully recovered.

The rare mouse that showed corneal opacity associated with band-shaped degeneration or neovascularization was excluded from further analysis.



Figure 3.2.1.2 Illustration of a homemade micropipette.

3.2.2 Measurement in the mouse model

3.2.2.1 Intraocular pressure

The rebound tonometer (Tonolab) utilizes a probe that lightly touches the cornea and measures rebound parameters to determine the mouse IOP, representing the most commonly used non-invasive method for mice.

For the AOH Mouse model:

At the 0-minute and 50-minute time points of the experiment, the mouse IOP was measured while a micropipette for hydrostatic pressure experiments remained inserted in the cornea. Mouse IOP was measured three consecutive times, with each measurement consisting of the probe touching the mouse cornea 6 times to obtain an average value.

For the SOH Mouse Model:

Mouse intraocular pressure measurements were performed without anesthesia to eliminate the influence of anesthetics on mouse IOP. A minimal amount of pupil-dilating agent (1% tropicamide solution, Mydracyl from Alcon) was topically applied to the corneal surface of silicone oil-filled eyes. Since IOP elevation is only detected after pupil dilation, we collected our IOP measurements 15 minutes after pupil dilation.

Mice were placed in a supine position throughout the measurement procedure, and all measurements were conducted between 9 and 10 AM to ensure consistency of IOP measurement.

Mouse IOP was measured twice weekly, with three measurements conducted each time.

The rebound tonometer device calculates a single measurement value by automatically computing the mean of six consecutive corneal contact assessments. IOP measurements were continuously

monitored for a duration of four weeks, after which subsequent experimental procedures were initiated.

3.2.2.2 Electroretinography

The mice underwent dark adaptation for 8-12 hours in advance. All procedures were conducted in a darkroom environment, using dim red light for experimental illumination. The mice were anesthetized deeply with an intraperitoneal injection of ketamine/xylazine (80 mg/kg:10 mg/kg body weight), and Lidocaine hydrochloride (20 mg/ml) was used for analgesia and anesthesia throughout the entire procedure. After anesthesia, 0.5% Tropicamide was promptly applied, followed by a 5-minute wait for full pupil dilation. Mice were positioned on a water-circulating heating pad to maintain their core temperature at 37°C.

A scotopic full-field ERG (ffERG) was used to measure retinal function in vivo. Applying gel to the surface of the mouse cornea before starting the experiment helps prevent corneal drying and ensures good contact with the electrode. During the experiment, avoid touching the electrodes by hand to prevent deformation, which could affect the accuracy of the experimental data. One platinum needle electrode (TE/S46-638) was inserted subcutaneously into the forehead between the two ears as a reference electrode, while another needle electrode was placed at the base of the tail as a ground electrode. A pair of gold ring electrodes (3mm diameter, RL-3103RC) was placed on the surface of the cornea to simultaneously capture light responses generated by both eyes as active electrodes. After positioning the four electrodes, the mouse was placed on the device platform and allowed to rest for 5 minutes before starting the experiment.

A Ganzfeld (Q450; RETI Animal, Roland Consult, Germany) was used to deliver light stimuli according to a customized protocol, with intensities ranging from $-5.10 \log \text{cd}\cdot\text{s}\cdot\text{m}^{-2}$ to $+1.3 \log \text{cd}\cdot\text{s}\cdot\text{m}^{-2}$. The stimulus data was collected by the RETI-Port system (Roland Consult). The positive scotopic threshold response (pSTR) and negative scotopic threshold response (nSTR) reflected the function of proximal retina, corresponding to retinal ganglion cells and amacrine cells, respectively. A wave reflected the function of photoreceptors, and the b wave reflected the function of bipolar cells. After completion of the ERG, the anesthetized animals will be placed on a heating pad and continuously monitored until they regain consciousness.

3.2.2.3 Optomotor test

Optomotor responses were measured using the OptoMotry System (OMR, CerebralMechanics, Canada), which presented drifting bars to mice through OptoMotry software on a gray background at 12.0 degrees per second and 0° orientation. To prevent the agitation that many mice display upon first encountering the testing platform, we implemented a pre-training protocol where mice were exposed to the OMR system for ~ 10 minutes on the day preceding the actual test.

Behavior tests, including contrast sensitivity and visual acuity, would be measured by this system. The contrast threshold for optomotor responses was established by starting with maximum contrast (100%) and manually reducing it until mice failed to demonstrate tracking behavior. Contrast sensitivity values were subsequently derived as the inverse of these threshold measurements at fixed spatial frequencies.

Experimental mice were placed on a holder platform positioned at the arena's center, enabling free standing and movement, while surrounded by a visual stimulus unit consisting of 4 LCD screens

displaying rotating stripes. Left and right eye responses were evaluated by measuring optomotor tracking in counterclockwise (CCW) and clockwise (CW) directions, respectively. Contrast sensitivity testing utilized 0.19 cycles per degree (cpd) as the spatial frequency parameter, selected for its documented ability to elicit excellent optomotor responses.

3.2.3 Retina preparation

Retina preparations were performed in dark-adapted conditions, which were for patch-clamp recordings and immunohistochemistry. The procedures were the same as mentioned above.

3.2.4 light stimulation

Light sensitivities of α RGCs were recorded under graded light intensities (2.9×10^1 to 1.2×10^5 Rh*/rod/s) using full-field green LED stimulation (525 nm) applied to the retinal surface.

3.2.5 Mechanical pressure on α RGCs

Two direct mechanical pressure methods were applied to isolate immediate effects on α RGC physiology: one using a pressure controller and another using homemade micropipette compression.

In the former method, controlled mechanical pressure was delivered to α RGCs through a homemade micropipette connected to a pressure controller (LEFOO, LFM512-OEC), elevating

intracellular pressure to simulate ocular hypertension. Each α RGC was exposed to sequential pressure applications of 40 Pa, 100 Pa, 200 Pa, and 300 Pa.

For the latter approach, α RGCs were directly compressed using a homemade micropipette to achieve a 15% soma diameter increase, replicating the pathological soma enlargement characteristic of glaucoma patients compared to normal controls (Liu et al., 2021).

3.2.6 Patch clamp

Loose patch recordings and whole cell recordings were performed on α RGCs from mid-peripheral regions of WT mouse retinas. Dye injections were applied to the KCNG-YFP mice, in which the labeled cells were easy to observe.

Spike recordings were collected and analyzed by Offline Sorter software (Plexon, Dallas, TX) and NeuroExplorer software (Nex Technologies, Littleton, MA). Peristimulus time Histograms (PSTHs) were constructed using NeuroExplorer's "PeriEvent Histogram" function to analyze the temporal spike frequency patterns (spikes/second) of single RGCs. Perievent raster plots were generated using NeuroExplorer to visualize the timing of light-evoked spikes relative to reference timestamps. Furthermore, NeuroExplorer enabled quantitative analysis of raster plots, which were then used to generate light response curves and determine α RGC light sensitivity.

3.2.7 Immunohistochemistry

Primary antibody for AII ACs used in this study was anti-Prox1 antibody (Rabbit, BioLegend, Cat#PRB-238C,1:500), and the secondary antibody is Cy3 anti-Rabbit IgG (Jackson

ImmunoResearch, Cat# 711-166-152, 1:200). Confocal images were acquired using a ZEISS LSM 800 confocal microscopy system with a pixel size of $0.156\ \mu\text{m} \times 0.156\ \mu\text{m}$.

For staining α RGCs, the SMI-32 (Biolegend, Cat# 201702, 1:500) was used in this study, and the secondary antibody is 488 anti-mouse IgG (Molecular Probes, Cat# A-21202, 1:200). The image size was $0.312\ \mu\text{m} \times 0.312\ \mu\text{m}$.

Image acquisition, analysis and statistical significance symbols were the same as mentioned above.

3.3 Results

3.3.1 IOP elevation of the SOH mouse model

To investigate the effects of sustained elevated IOP on the retina, we established a mouse model of elevated IOP using silicone oil. Silicone oil was injected into the anterior chamber of WT mice using a sharp micropipette (**Fig. 3.3.1A**). The silicone oil blocked the pupil (**Fig. 3.3.1B**). The IOP of a single mouse was continuously monitored over the following 4 weeks (**Fig. 3.3.1C**). The IOP began to rise the day after the silicone oil injection, increasing from 7.67 ± 0.33 mmHg to 29.33 ± 1.33 mmHg, and subsequently stabilized. The IOP was 33.67 ± 1.33 mmHg in the first week, 31.33 ± 1.76 mmHg in the second week, 31.33 ± 0.67 mmHg in the third week, and 24 ± 1.0 mmHg in the fourth week. The IOP data from all experimental mice were recorded and analyzed (**Fig. 3.3.1D**). The initial IOP of mice was 9.79 ± 0.25 mmHg, which significantly increased to 29.52 ± 1.96 mmHg on the second day, 36.26 ± 2.34 mmHg in the second week, and 34.15 ± 2.43 mmHg in the fourth week.

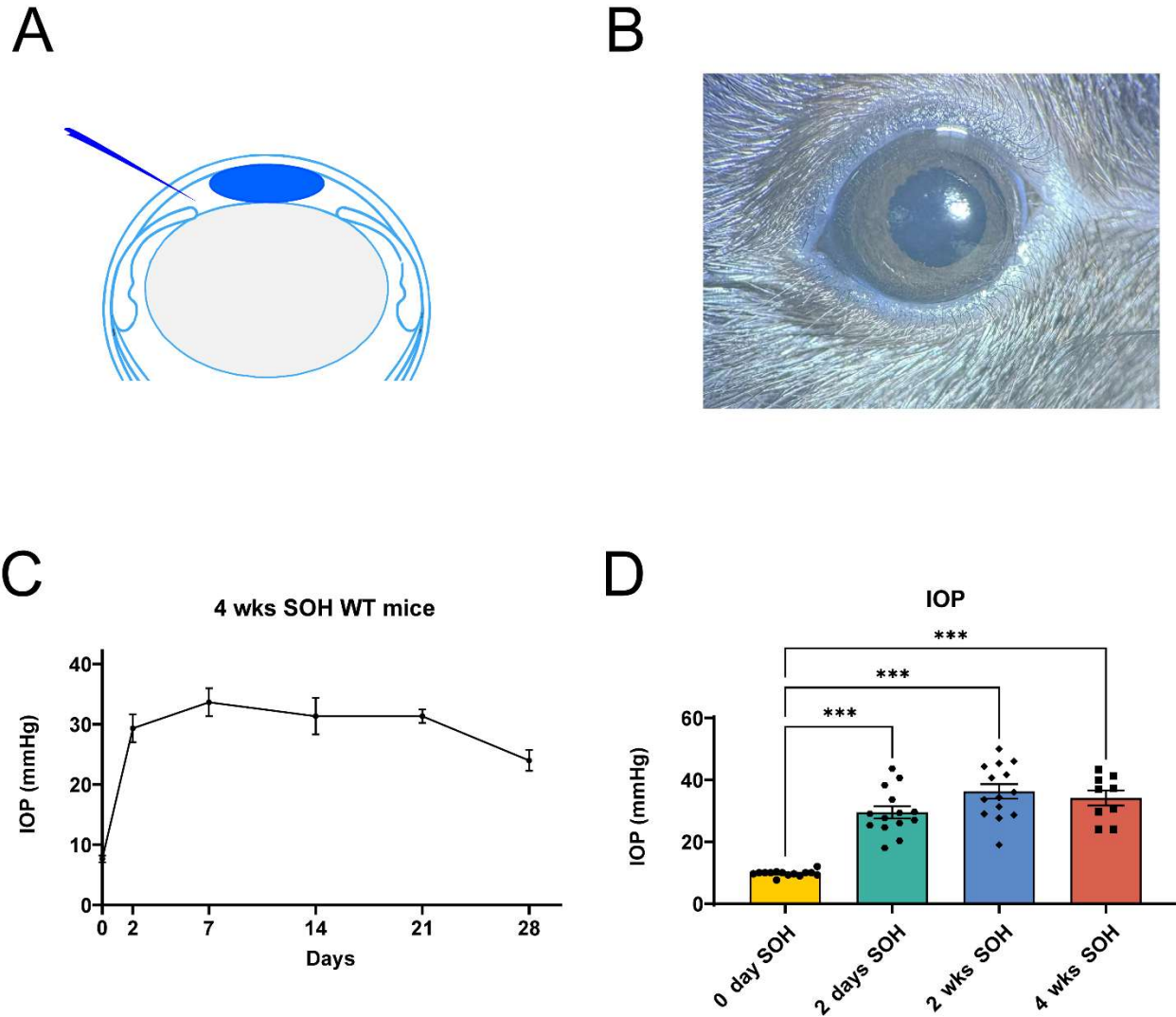


Figure 3.3.1 IOP elevation of the SOH mouse model

(A) Schematic representation of the SOH mouse model.

(B) Representative image of the anterior chamber in a mouse after silicone oil injection.

(C) IOP changes in a single mouse for over four weeks after silicone oil injection.

(D) Continuous IOP measurements over 4 weeks SOH injury. (Control: $n = 14$; 2 days SOH eye: $n = 14$; 2 wks SOH eye: $n = 14$; 4 wks SOH eye, $n = 9$. These data are Mean $\pm$ SEM. Statistical analysis: one-way ANOVA with Tukey's multiple comparisons: ***, $p < 0.001$.)

3.3.2 Decline of inner retinal function in the SOH mouse model

To assess the retinal function after the 4 weeks SOH injury, the scotopic full-field electroretinography (ffERG) was conducted to record the mass voltage signal generated by retinal cells using light flash stimuli. The distinct ERG waveform characteristics exhibited by the light responses of different retinal cells under varying light intensities can be used to roughly distinguish between cell types. In this experiment, the WT mice were pre-adapted to darkness for 12 hours. A light intensity of $-4.05 \log \text{cd} \cdot \text{s} \cdot \text{m}^{-2}$ was used to record positive scotopic threshold response (pSTR) (**Fig. 3.3.2A**) and negative scotopic threshold response (nSTR), while an intensity of $+1.30 \log \text{cd} \cdot \text{s} \cdot \text{m}^{-2}$ was applied to elicit a-wave and b-wave responses (**Fig. 3.3.2B**). pSTR and nSTR primarily reflect the activity of retinal ganglion cells and the contribution of amacrine cells, respectively. The function of photoreceptors can be represented by the a-wave, while the b-wave is primarily attributed to the activity of bipolar cells. At 4 weeks, no statistically significant difference was observed in the maximum response amplitudes of the a-wave and b-wave between the control group (a wave: $-391.6 \pm 12.89 \mu\text{V}$, $n = 6$; b wave: $679.3 \pm 26.57 \mu\text{V}$, $n = 6$) and the SOH group (a wave: $-348.1 \pm 23.10 \mu\text{V}$, $n = 6$; b wave: $597.4 \pm 56.08 \mu\text{V}$, $n = 6$) (**Fig. 3.3.2C**). Compared to the control group (nSTR: $-49.72 \pm 2.24 \mu\text{V}$, $n = 6$; pSTR: $86.13 \pm 4.17 \mu\text{V}$, $n = 6$), the response amplitudes of pSTR and nSTR were statistically reduced in the SOH group (nSTR: $-38.33 \pm 3.66 \mu\text{V}$, $n = 6$; pSTR: $57.35 \pm 9.17 \mu\text{V}$, $n = 6$) (**Fig. 3.3.2D**).

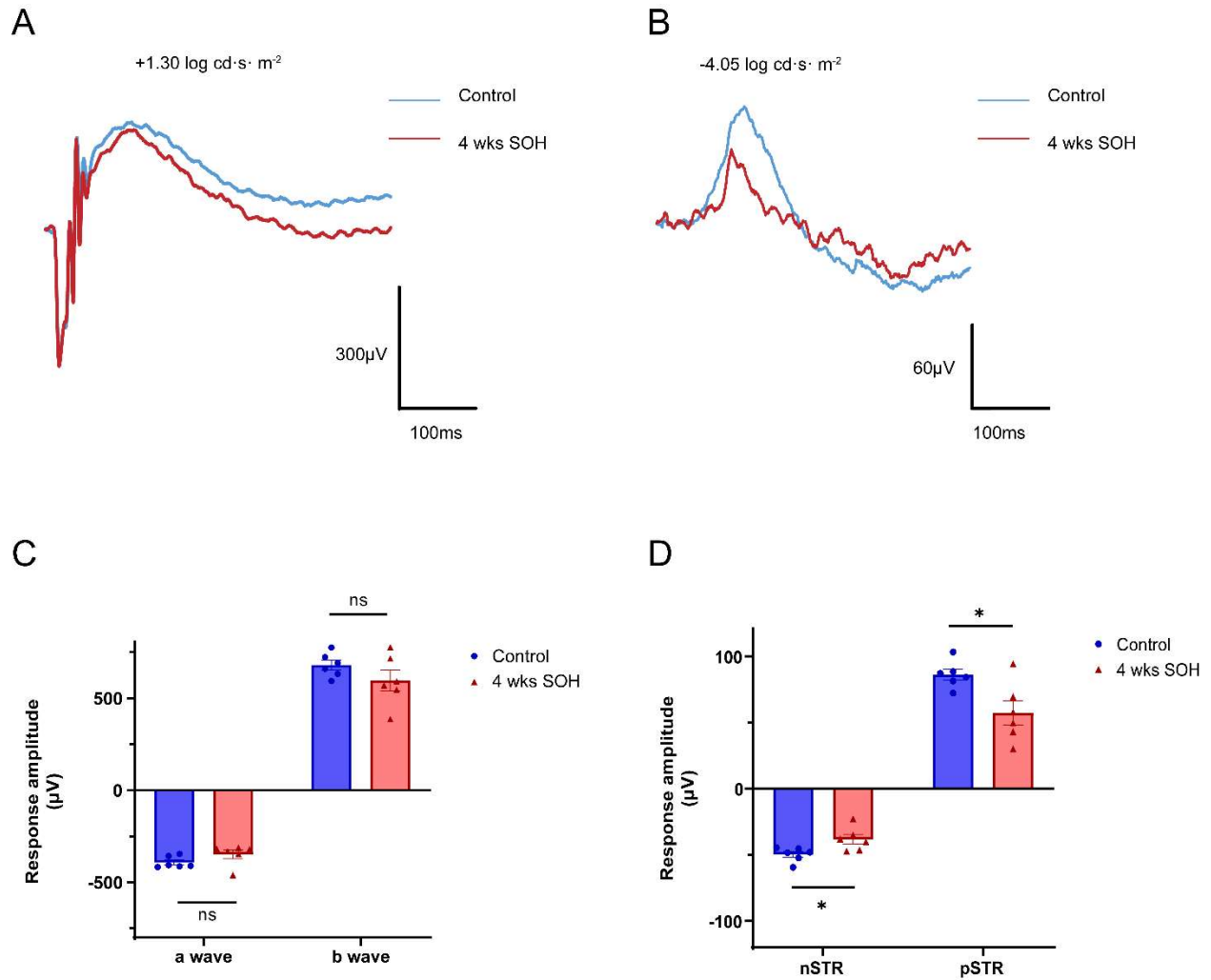


Figure 3.3.2 ERG responses of control and 4-week SOH mice.

(A, B) Representative ERG waveforms of control mice(blue) and 4-week SOH mice(red) at two different stimulus intensities. Responses from $+1.30 \log \text{cd} \cdot \text{s} \cdot \text{m}^{-2}$ represent a wave and b wave, while those from $-4.05 \log \text{cd} \cdot \text{s} \cdot \text{m}^{-2}$ perform nSTR and pSTR.

(C, D) The comparison of response amplitudes of a wave, b wave, pSTR, and nSTR between control and 4-week SOH mice. These data are mean $\pm$ SEM. Statistical analysis: a wave (Mann-Whitney test), b wave (unpaired t test), pSTR (unpaired t test), nSTR (unpaired t test).

3.3.3 The loss of α RGCs in the retina of 4-week SOH mice

To examine changes in α RGCs and AII ACs following 4 weeks of SOH, immunostaining was performed on whole-mount retinal preparations in WT mice. Retinas were flat-mounted and stained with SMI-32 for α RGCs (**Fig. 3.3.3A-C**) and Prox1 for AII ACs (**Fig. 3.3.3D-F**). The labeled cells were quantified to assess their numbers and distribution. The retina was divided into three regions: the far-peripheral region, mid-peripheral region, and central region.

The number of SMI-32 positive cells in the 4-week SOH group were significantly reduced compared to the control group in the far-peripheral region (control group: 21.88 ± 0.46 cells/field, $n = 6$; 4 wks SOH group: 14.29 ± 0.97 cells/field, $n = 6$), mid-peripheral region (control group: 28.17 ± 0.53 cells/field, $n = 6$; 4 wks SOH: 19.46 ± 1.0 cells/field, $n = 6$) and central region (control: 31.79 ± 0.60 cells/field, $n = 6$; 4 wks SOH: 26.54 ± 1.21 cells/field, $n = 6$). Quantitative analysis showed that the number of prox1-positive cells did not change after 4 weeks of silicone oil injection compared to the control group in the far-peripheral region (control group: 59.06 ± 1.87 cells/field, $n = 4$; 4 wks SOH group: 56.50 ± 2.57 cells/field, $n = 4$), mid-peripheral region (control group: 88.88 ± 2.80 cells/field, $n = 4$; 4 wks SOH: 90.19 ± 3.16 cells/field, $n = 4$) and central region (control: 104.50 ± 3.57 cells/field, $n = 4$; 4 wks SOH: 105.90 ± 2.89 cells/field, $n = 4$).

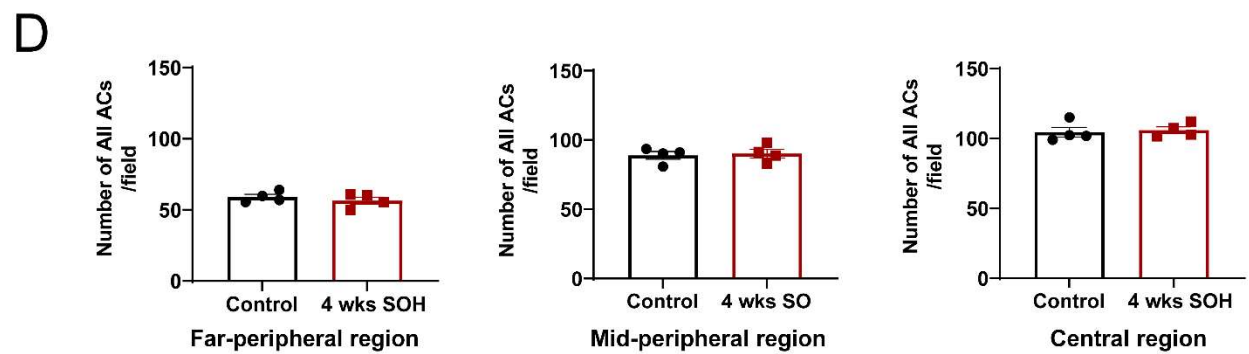
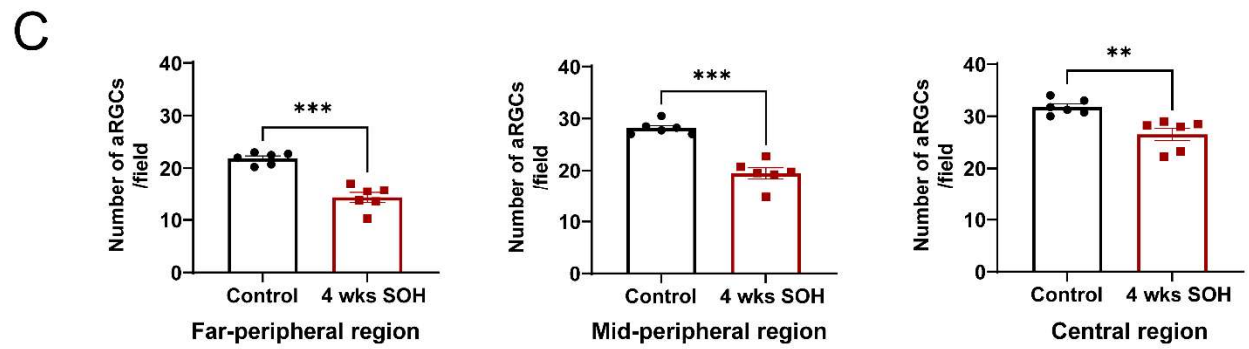
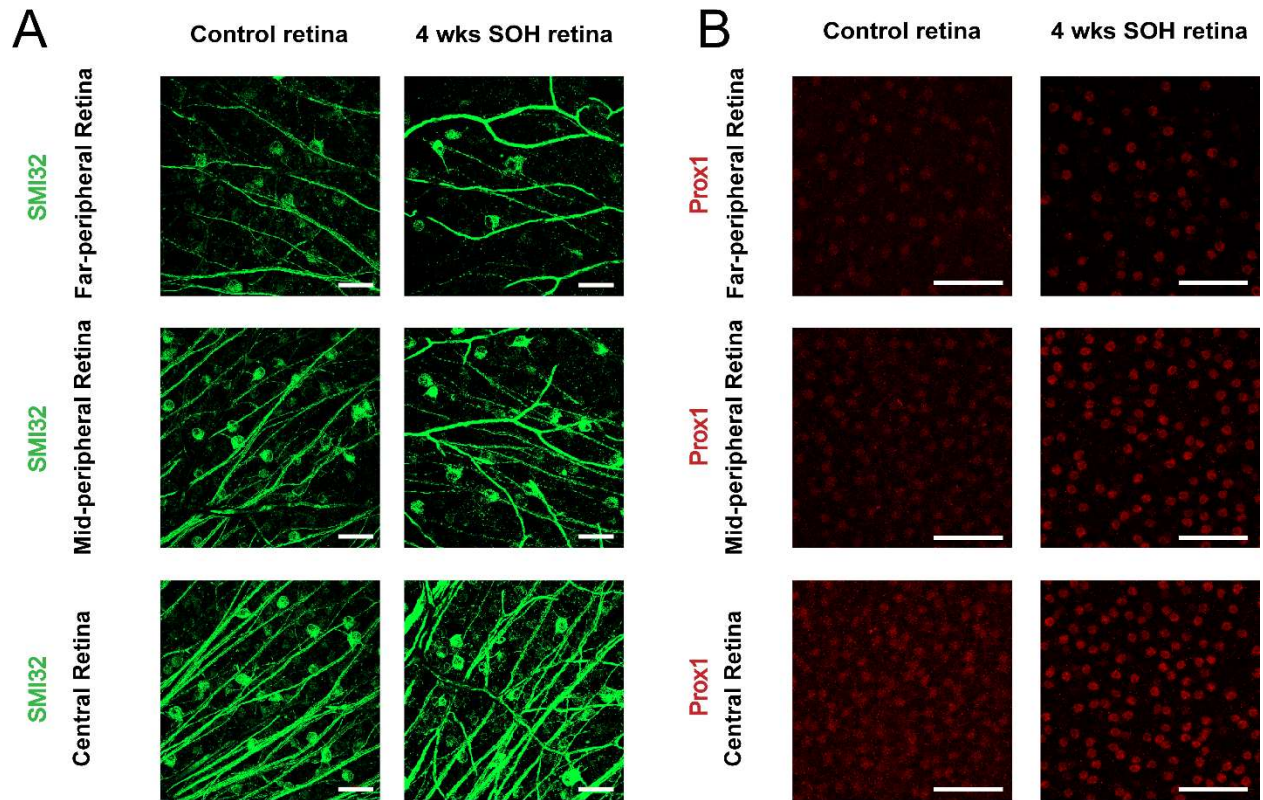


Figure 3.3.3 The number of RGCs, AII ACs of control and 4 weeks of SOH mice.

(A) Representative images of immunostained whole-mount α RGCs from the control group and the 4-week SOH group in the far-peripheral retinal regions, mid-peripheral retinal regions, and central retinal regions. The cells were stained with SMI-32 (Green). Scale bar: 50 μ m.

(B) Representative images of immunostained whole-mount AII ACs from the control group and the 4-week SOH group in the far-peripheral retinal regions, mid-peripheral retinal regions, and central retinal regions. The cells were stained with Prox1 (Red). Scale bar: 50 μ m.

(C, D) Quantitative analysis of the α RGCs and AII ACs was conducted in the control group and the 4-week SOH group across three different regions.

(These data are mean $\pm$ SEM. Statistical analysis: one-way ANOVA with multiple comparisons)

3.3.4 The reduced light sensitivities of four subtypes of α RGCs in mice following 4 weeks of SOH

The retinas were illuminated by a 525nm wavelength green light at gradually increasing intensities, ranging from 2.9×10^1 to 1.2×10^5 Rh*/rod second. Light-evoked spike responses of four kinds of α RGCs subtypes were recorded extracellularly with loose-patch electrodes in WT mice. Raster plots illustrated the diverse light-responsive patterns of α RGCs, enabling the classification of ON transient α RGC (**Fig. 3.3.4A**), ON sustained α RGC (**Fig. 3.3.4D**), OFF transient α RGC (**Fig. 3.3.4G**), and OFF sustained α RGC (**Fig. 3.3.4J**).

The normalized light spikes were plotted against the light intensities and fitted by the Hill equation. Light response thresholds were determined as the illumination level eliciting 5% of the peak response amplitude. In representative ON transient α RGCs (**Fig. 3.3.4B**) and ON sustained

α RGCs (Fig. 3.3.4E), compared with the control group, the normalized R-LogI curves shifted significantly to the right after 4-week SOH injury. The light intensity threshold increased from 7.94×10^1 to 5.89×10^3 Rh*/rod second in ON transient α RGCs and from 1.58×10^3 to 5.83×10^3 Rh*/rod second in ON sustained α RGCs. Quantitative results indicated that light thresholds of ON transient α RGCs (**Fig. 3.3.4C**) and ON sustained α RGCs (**Fig. 3.3.4F**) are significantly elevated in SOH mice (ON transient: $2.26 \pm 0.01 \times 10^4$ Rh*/rod sec, $n = 4$; ON sustained: $7.29 \pm 0.01 \times 10^3$ Rh*/rod second, $n = 3$) compared to control mice (ON transient: $1.37 \pm 0.02 \times 10^2$ Rh*/rod second, $n = 4$; ON sustained: $1.85 \pm 0.02 \times 10^2$ Rh*/rod second, $n = 6$). A similar phenomenon was observed in OFF transient α RGCs (**Fig. 3.3.4H**) and OFF sustained α RGCs (**Fig. 3.3.4K**). Four weeks after SOH injury, the curves of these two types of α RGCs were observed to shift rightward compared to those of the control group. The light intensity threshold increased from 1.00×10^3 to 3.57×10^3 Rh*/rod second in OFF transient α RGCs and from 3.98×10^2 to 4.29×10^3 Rh*/rod second in OFF sustained α RGCs. Statistical analysis suggested a significant increase in the light intensity thresholds of OFF transient α RGCs and OFF sustained α RGCs in the four-week group (OFF transient: $8.61 \pm 0.01 \times 10^3$ Rh*/rod second, $n = 4$; OFF sustained: $6.76 \pm 0.01 \times 10^2$ Rh*/rod second, $n = 4$) compared to the control group (OFF transient: $1.77 \pm 0.01 \times 10^3$ Rh*/rod second, $n = 4$; OFF sustained: $4.73 \pm 0.01 \times 10^3$ Rh*/rod second, $n = 5$).

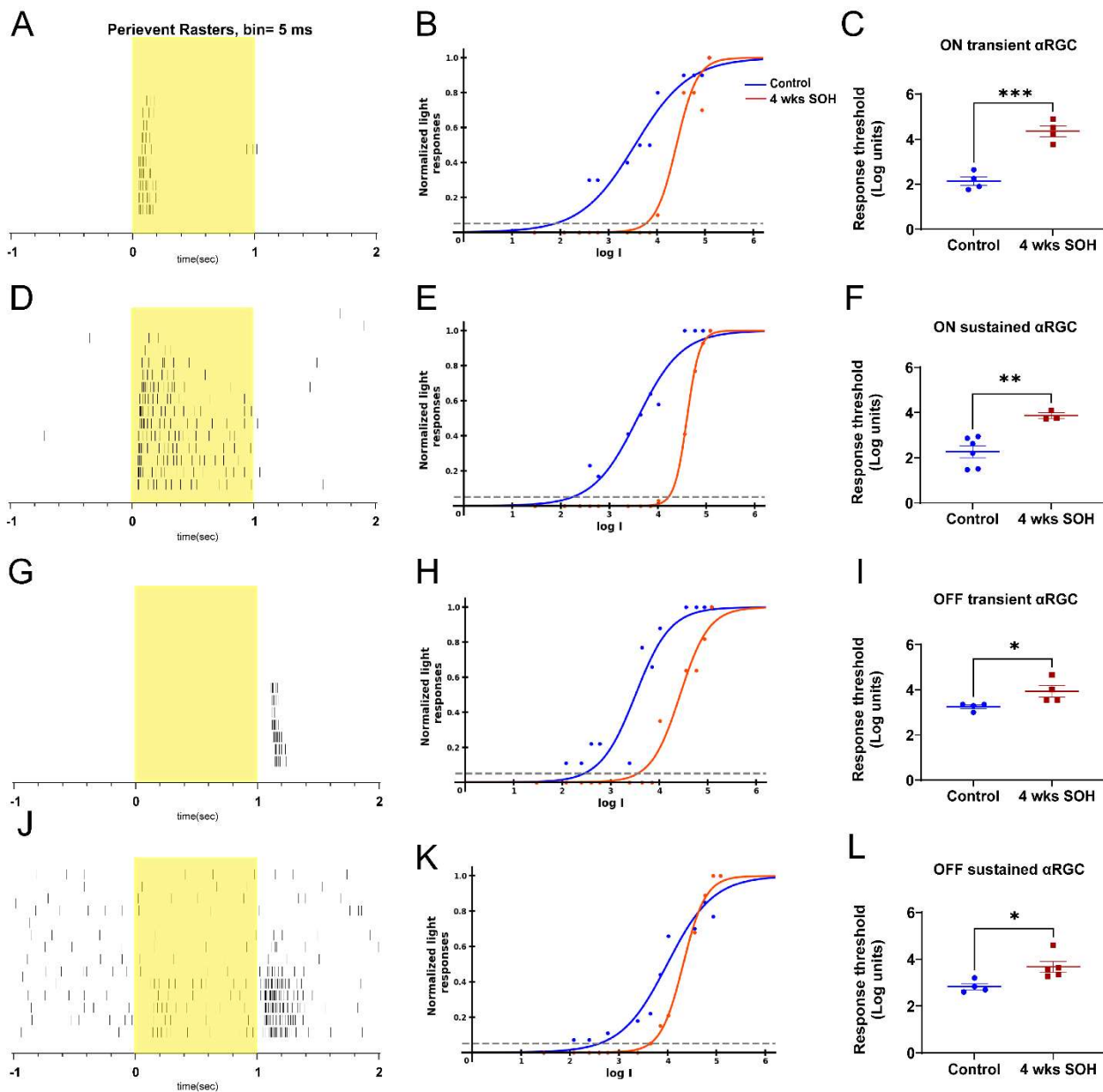


Figure 3.3.4 The effect of 4-week SOH on the light intensity of four subtypes of α RGCs.

(A, D, G, J) Raster plots of light-evoked spikes of an ON transient, ON sustained, OFF transient, and OFF sustained α RGC at steadily increasing light intensities in the control group (bin=5ms).

(B, E, H, K) The representative normalized R-LogI curves of four types of α RGCs of control mice and 4-week SOH mice (blue: control mice; red: 4-week SOH mice).

(C, F, I, L) Light response thresholds for the four α RGC types of control mice and 4-week SOH mice.

(These data are Mean $\pm$ SEM. Statistical analysis: unpaired t-test.)

3.3.5 Elevated IOP and decreased contrast sensitivity in the AOH mouse model

The hydrostatic pressure-induced IOP elevation was characterized as an acute phenomenon, exclusively confined to the duration of the experimental procedure (**Fig. 3.3.5A**). In this experiment, the magnitude of WT mouse IOP elevation was predominantly determined by the vertical height of the water column. The present investigation employed a column height of 1 meter, corresponding to an intraocular pressure of approximately 75 mmHg. The IOP increased from 14.00 ± 1.14 mmHg at the start of the experiment to 74.2 ± 6.45 mmHg, maintaining a state of elevated ocular pressure throughout the duration of the experiment. At the 50-minute mark, the IOP was recorded at 72.00 ± 3.80 mmHg. Following the experiment, the IOP of the mice was monitored, showing a return to normal levels. Specifically, IOP was observed to be 11.50 ± 0.65 mmHg at 100 minutes, 11.00 ± 0.58 mmHg at 150 minutes, 14.50 ± 1.50 mmHg at 2 weeks, and 13.00 ± 1.16 mmHg at 4 weeks (**Fig. 3.3.5B**).

To investigate whether the contrast sensitivity of mice have changed, we used the optomotor response to conduct behavioral tests evaluating their visual function. Testing of the mice was initiated one day after the experiment to minimize the impact of post-experimental corneal edema on their optomotor response. The contrast sensitivity of the mice decreased from 13.35 ± 0.91 to 13.00 ± 1.23 one day after hydrostatic pressure, although this was not a significant difference. It further dropped to 9.86 ± 0.92 after two weeks and showed a significant difference by decreasing to 6.22 ± 0.76 after four weeks (**Fig. 3.3.5C**).

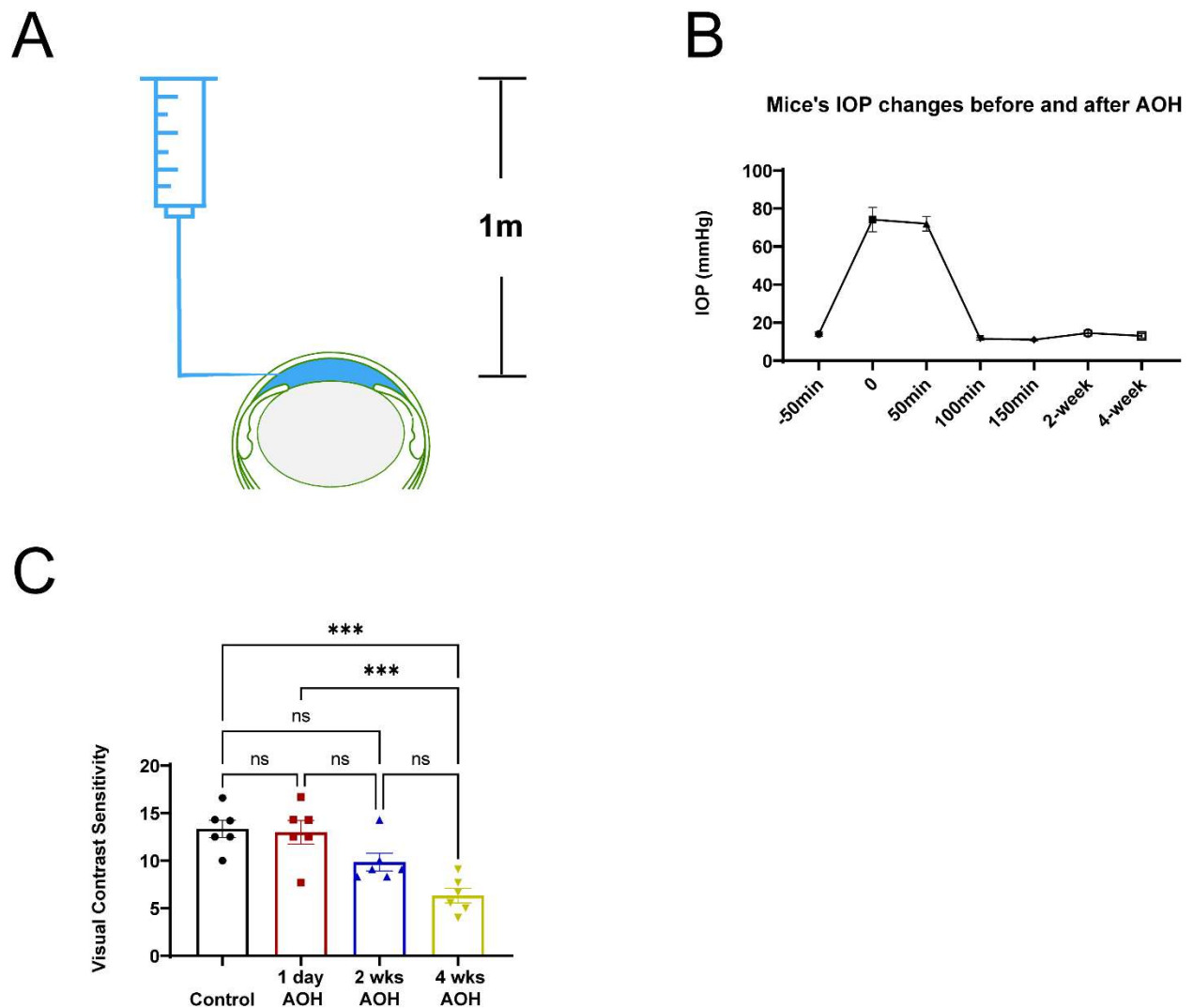


Figure 3.3.5 IOP measurement and optomotor response after AOH injury.

(A) Schematic representation of the AOH mouse model.

(B) Representative WT mouse's IOP changed before and after AOH injury.

(C) Visual contrast sensitivity of AOH mouse models in the control group (n=6), 1-day group (n=6), 2-week group (n=6), and 4-week group (n=6).

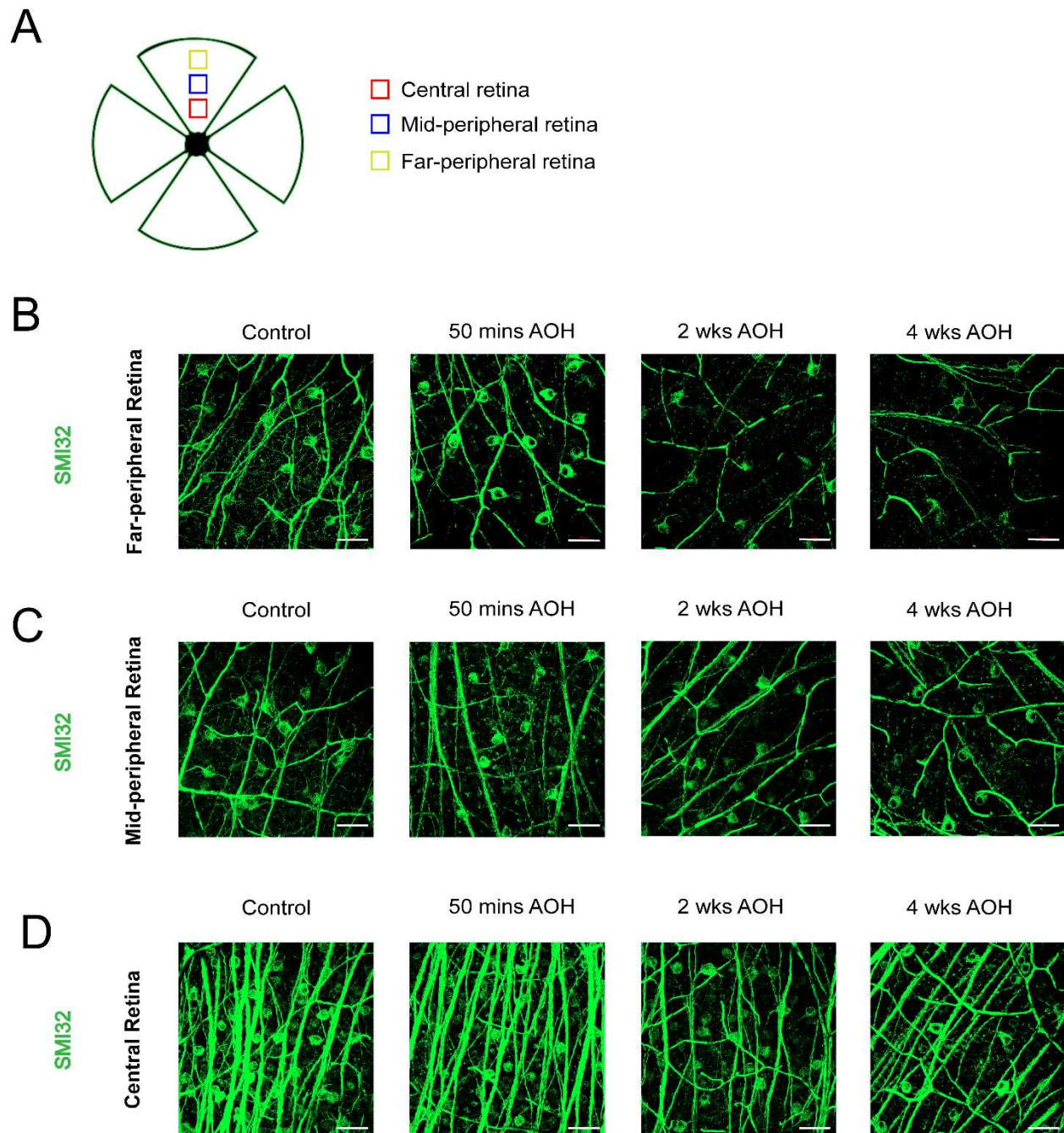
3.3.6 AOH injury results in the loss of the α RGCs

To examine the effects of AOH on the retinal neurons, we analyzed quantitative changes in α RGCs over a four-week period following AOH injury. We divide the retina into three regions based on its distance from the optic nerve: the far-peripheral region, mid-peripheral region, and central region (**Fig. 3.3.6A**). SMI-32 is used to label α RGCs, and the control group, 50-minute group, 2-week group, and 4-week group will be used for comparison.

In the far-peripheral region (**Fig. 3.3.6B, E**), the number of SMI 32-positive immunoreactive cells remained unchanged after 50 minutes of AOH, decreasing only slightly from 21.88 ± 0.46 cells/field in the control group to 21.25 ± 0.46 cells/field. It significantly declined to 16.33 ± 0.26 cells/field after 2 weeks and continued to decrease markedly to 11.08 ± 0.61 cells/field after 4 weeks.

In the mid-peripheral region (**Fig. 3.3.6C, F**), quantitative analysis showed that the number of SMI 32-positive cells did not change after 50 minutes of hydrostatic pressure compared to the control group (28.17 ± 0.53 cells/field and 28.17 ± 0.71 cells/field, respectively). The count substantially decreased to 19.79 ± 0.79 cells/field after 2 weeks and continued to decline to 16.54 ± 0.78 cells/field after 4 weeks.

In the central region (**Fig. 3.3.6D, G**), the number of SMI 32+ cells remained stable after 50 minutes of hydrostatic pressure (31.79 ± 0.60 cells/ field and 31.63 ± 0.24 cells/ field, respectively). A significant reduction to 23.83 ± 0.40 cells/field was observed after two weeks, and the count further declined markedly to 19.96 ± 0.68 cells/field after four weeks.



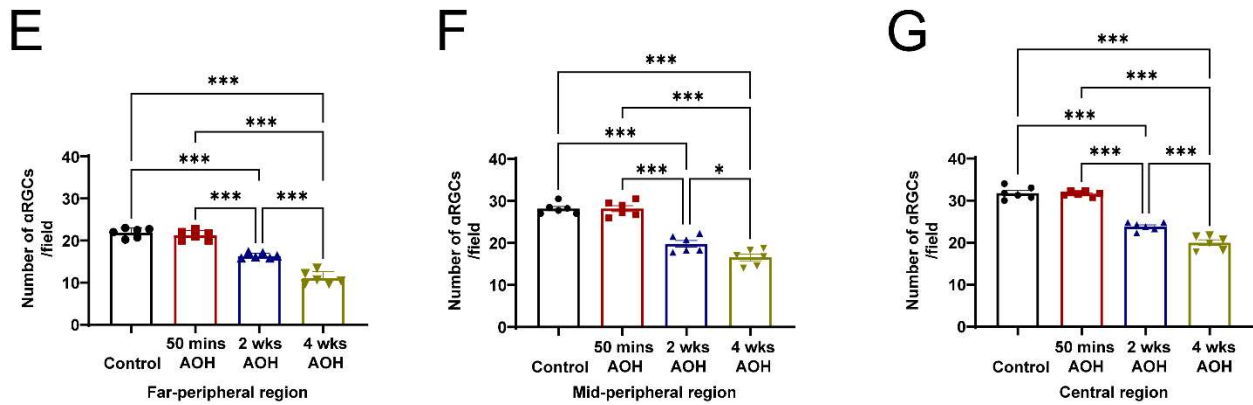


Figure 3.3.6 The quantitative analysis of α RGCs in the retinas of WT mice following AOH at 50 minutes, 2 weeks, and 4 weeks post-elevation.

(A) Schematic diagram of location selection for confocal microscopy in the retina. Confocal microscopy sampling sites are distributed across three defined regions within each of the four retinal quadrants.

(B-D) Representative images of immunostaining whole-mount from the control group, 50 mins group, 2 weeks group, and 4 weeks group in the far-peripheral retinal regions, mid-peripheral retinal regions, and central retinal regions. The cells were stained with SMI-32. Scale bar: 50 μ m.

(E-G) A quantitative analysis of the α RGC count was conducted across three different regions, including the control group, the group subjected to 50 minutes of AOH injury, the group subjected to two weeks of AOH, and the group subjected to four weeks of AOH.

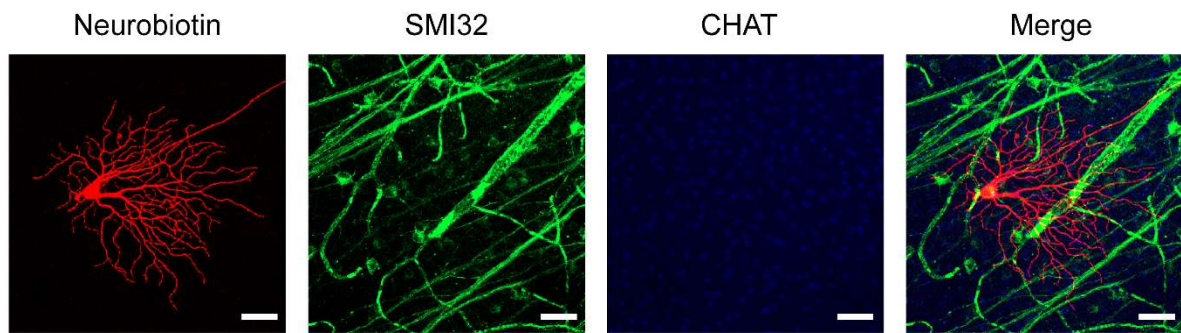
(These data are mean $\pm$ SEM. Statistical analysis: one-way ANOVA with multiple comparisons.)

3.3.7 Unchanged dendritic field and soma size of α RGCs following AOH at 50 minutes, 2 weeks, and 4 weeks post-elevation

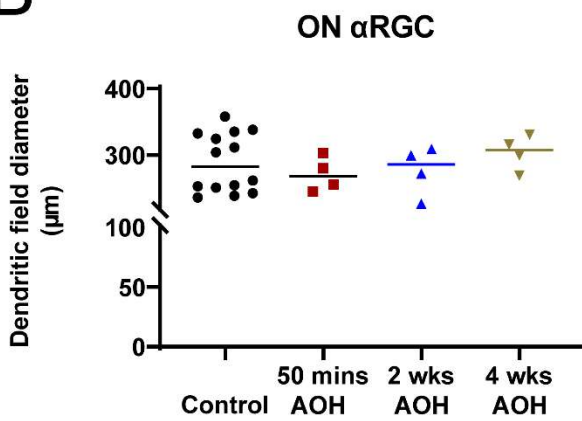
To assess the impact of AOH on the morphology of ON and OFF α RGCs, we focused on two critical features of neuronal structure: the soma diameter and the dendritic field diameter. NB was utilized to visualize cellular morphology, SMI-32 to specifically identify α RGCs, and ChAT to distinguish between ON and OFF subtypes (**Fig. 3.3.7A**).

Acute ocular hypertension within 4 weeks did not affect the dendritic field diameter (Control: $288.40 \pm 11.81 \mu\text{m}$, $n = 14$; 50 minutes: $270.60 \pm 13.01 \mu\text{m}$, $n = 4$; 2 weeks: $276.60 \pm 18.45 \mu\text{m}$, $n = 4$; 4 weeks: $303.50 \pm 13.22 \mu\text{m}$, $n = 4$) or soma diameter (Control: $20.37 \pm 0.43 \mu\text{m}$, $n = 14$; 50 minutes: $22.23 \pm 0.95 \mu\text{m}$, $n = 4$; 2 weeks: 20.29 ± 0.68 , $n = 4$; 4 weeks: $22.22 \pm 0.79 \mu\text{m}$, $n = 4$) of ON α RGCs (**Fig. 3.3.7B, C**). There was no statistically significant difference in the dendritic field diameter (Control: $269.70 \pm 11.50 \mu\text{m}$, $n = 7$; 50 minutes: $307.5 \pm 11.57 \mu\text{m}$, $n = 4$; 2 weeks: $282.10 \pm 9.72 \mu\text{m}$, $n = 4$; 4 weeks: $252.50 \pm 6.08 \mu\text{m}$, $n = 7$) or soma diameter (Control: $21.39 \pm 0.86 \mu\text{m}$, $n = 7$; 50 minutes: $21.95 \pm 1.72 \mu\text{m}$, $n = 4$; 2 weeks: $20.53 \pm 0.47 \mu\text{m}$, $n = 4$; 4 weeks: $19.92 \pm 0.66 \mu\text{m}$, $n = 7$) of OFF α RGCs within the following 4 weeks. In summary, AOH injury did not noticeably affect the morphologies of ON and OFF α RGCs during the subsequent 4 weeks (**Fig. 3.3.7D, E**).

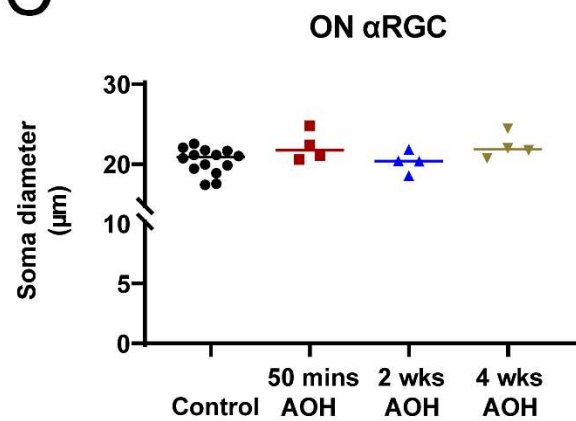
A



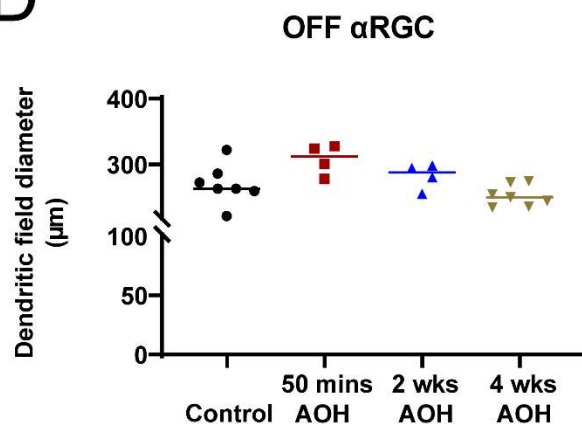
B



C



D



E

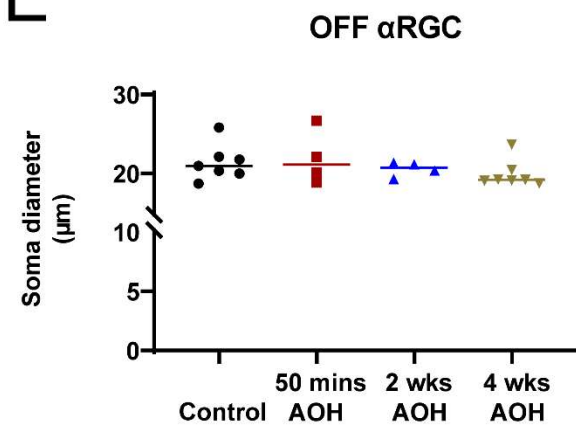


Figure 3.3.7 The morphology of ON and OFF α RGCs in the retinas of WT mice following AOH at 50 minutes, 2 weeks, and 4 weeks post-elevation.

(A) Representative images of immunostaining morphology of α RGC. NB labeled cells in red, SMI-32 in green, and ChAT in blue. Scale bar: 50 μ m.

(B, C) Dendritic field diameter and soma diameter of ON α RGCs in the control group (n=14), 50-minute group (n=4), 2-week group (n=4), and 4-week group (n=4).

(D, E) Dendritic field diameter and soma diameter of OFF α RGCs in the control group (n=7), 50-minute group (n=4), 2-week group (n=4), and 4-week group (n=7).

(These data are mean $\pm$ SEM. Statistical analysis: one-way ANOVA with multiple comparisons.)

3.3.8 After four weeks AOH, there was a significant decrease in the maximum light response of α RGCs

To further investigate whether AOH injury for 2 weeks and 4 weeks affects the excitatory and inhibitory input signals of RGCs, we employed the whole-cell patch-clamp technique. Cells were voltage-clamped at -68 mV for excitatory postsynaptic currents (EPSCs) and +20 mV for inhibitory postsynaptic currents (IPSCs) to examine their responses to varying light intensities.

Fig. 3.3.8A presents representative EPSCs recorded at -68 mV under 1-second illumination with varying light intensities at 525 nm, obtained from the same ON α RGC.

The light-evoked EPSC threshold of ON α RGCs shifted from 2.3 to 4.67×10^2 Rh*/rod sec after 2 weeks of hydrostatic pressure and further shifted to 3.5 after 4 weeks in **Fig. 3.3.8B**. Statistical analysis in **Fig. 3.3.8C** revealed no significant difference in the threshold between the control

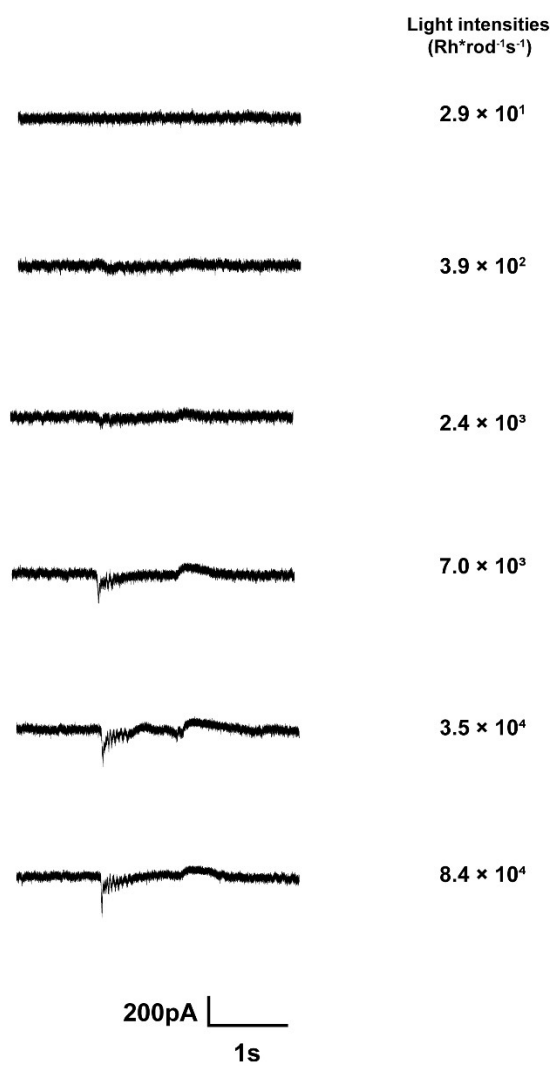
group (2.36 ± 0.23) and the 2-week AOH group (2.52 ± 0.15), but a significant increase was observed in the 4-week group (3.60 ± 0.17) compared to the control.

The light-evoked IPSC curve of ON α RGCs exhibited a continuous rightward shift after AOH injury, with the threshold moving from the baseline of 2.3 to 2.5 after 2 weeks and reaching 3.5 after 4 weeks (**Fig. 3.3.8D**). While the threshold in the 2-week AOH group (2.63 ± 0.15) showed a slight increase compared to the control (2.50 ± 0.11), the difference was not statistically significant. A significant elevation was observed in the 4-week group (3.66 ± 0.26) compared to the control (**Fig. 3.3.8E**).

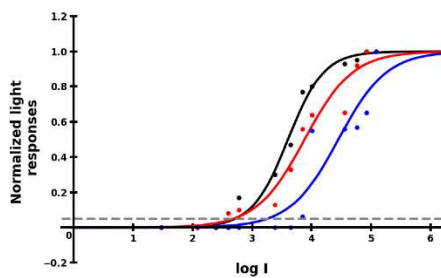
A similar trend was observed in the light response of OFF α RGCs. The light-evoked EPSC curve of OFF α RGCs demonstrated a gradual rightward shift following AOH injury. The threshold rose from a baseline of 2.3 to 2.5 at 2 weeks and reached 3.5 after 4 weeks (**Fig. 3.3.8F**). While the 2-week group (2.26 ± 0.10) showed a modest upward shift in the threshold compared to the control (2.20 ± 0.07), this change was not statistically significant. By 4 weeks, however, the threshold (3.26 ± 0.36) was significantly elevated relative to the control group (**Fig. 3.3.8G**).

The light-evoked IPSC curve of OFF α RGCs showed a progressive rightward shift under AOH injury. After 2 weeks, the threshold shifted from the baseline value of 2.3 to 2.5, and by 4 weeks, it had further increased to 3.5 (**Fig. 3.3.8H**). While the 2-week AOH group (2.1 ± 0.19) displayed a mildly elevated threshold compared to the control (2.01 ± 0.25), the difference was not statistically significant. In contrast, the 4-week group (3.36 ± 0.25) demonstrated a significantly higher threshold compared to the control (**Fig 3.3.8I**).

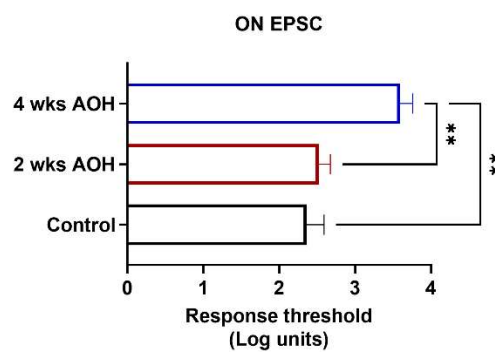
A



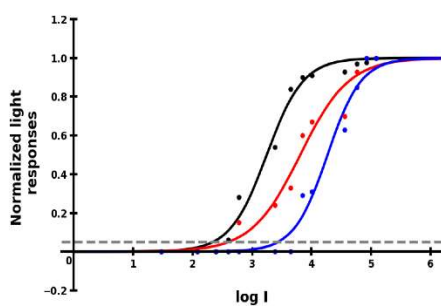
B



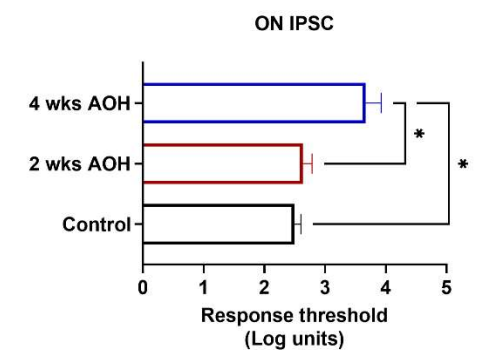
C



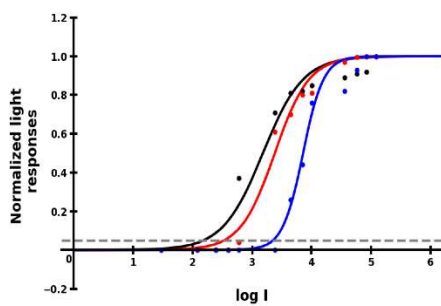
D



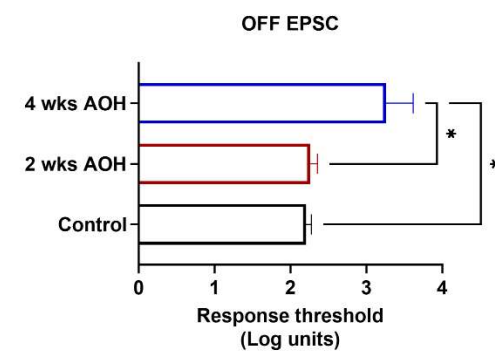
E



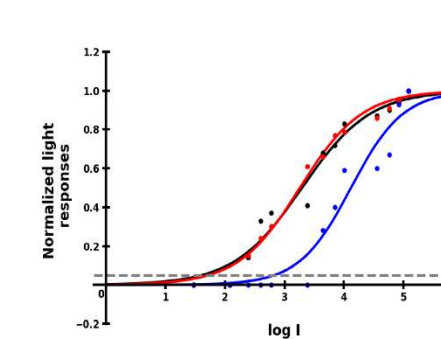
F



G



H



I

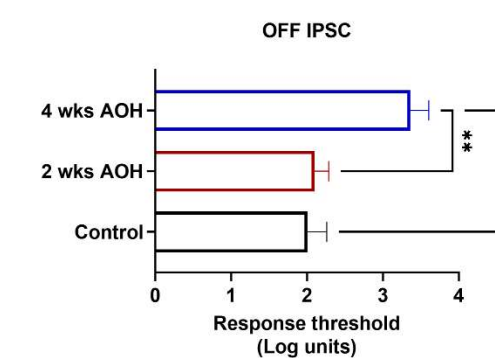


Figure 3.3.8 Light response after AOH.

(A) Representative image of light-evoked EPSC amplitudes recorded at -68 mV from ON α RGCs under different light intensities, including 2.9×10^1 Rh*/rod sec, 3.9×10^2 Rh*/rod sec, 2.4×10^3 Rh*/rod sec, 7.0×10^3 Rh*/rod sec, 3.5×10^4 Rh*/rod sec, 8.4×10^4 Rh*/rod sec.

(B, D, F, H) Fitted normalized response-intensity curves for EPSCs and IPSCs of ON RGC and OFF RGC in the control group (black), 2 weeks AOH group (red), and 4 weeks AOH group (blue).

(C, E, G, I) Light response thresholds for EPSCs and IPSCs of ON RGC and OFF RGC in the control group (black), 2 weeks AOH group (red), and 4 weeks AOH group (blue). Average thresholds are defined as the light intensity eliciting 5% of the maximum spike response.

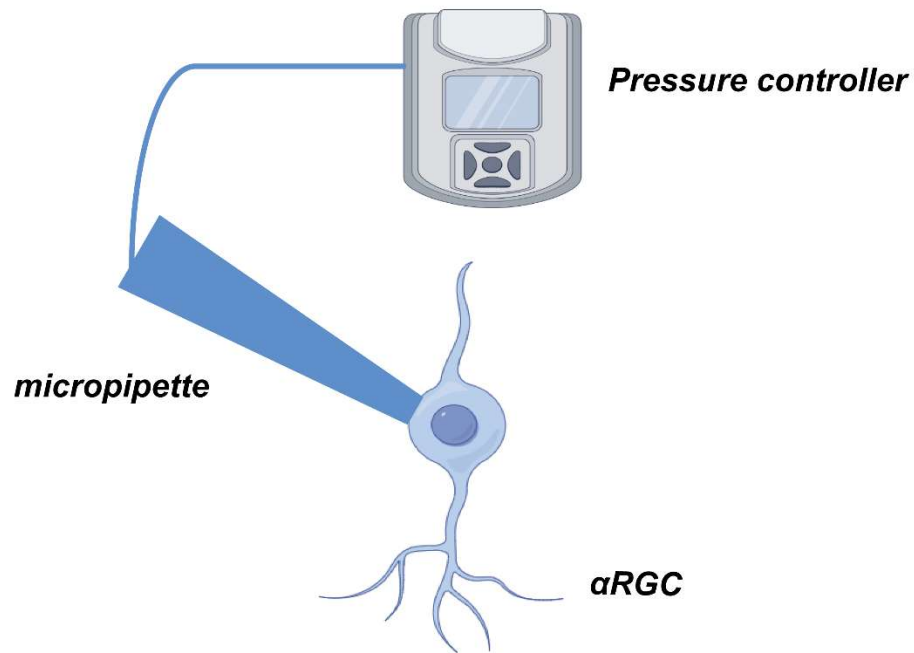
(These data are mean $\pm$ SEM. Statistical analysis: one-way ANOVA with multiple comparisons; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.)

3.3.9 Increase in α RGC spikes under direct mechanical pressure applied by pressure controller

To elucidate whether direct pressure exerts any effects on α RGCs, we employed a pressure controller connected to a micropipette to directly apply controlled pressure to individual α RGCs in WT mice (**Fig. 3.3.9A**). Pressures of 40 Pa, 100 Pa, 200 Pa, and 300 Pa were sequentially applied to the same α RGC using a pressure controller. The spike frequency and spike count were analyzed using NeuroExplorer software. Under increasing pressure, the spike frequency generally increased gradually. Meanwhile, although light-evoked spikes were detectable, the peak of the light response remained within the 95% confidence interval (**Fig. 3.3.9B-F**). This suggests that the pressure-induced increase in spikes may interfere with the detection of light signals of α RGCs. Compared

to the control group, the normalized R-LogI curve shifted to the right after the increase in pressure, indicating a reduction in cellular light sensitivity (**Fig. 3.3.9G**).

A



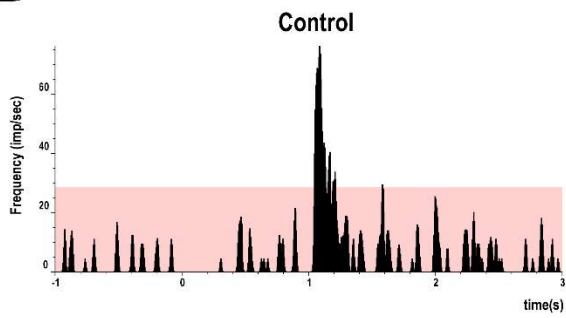
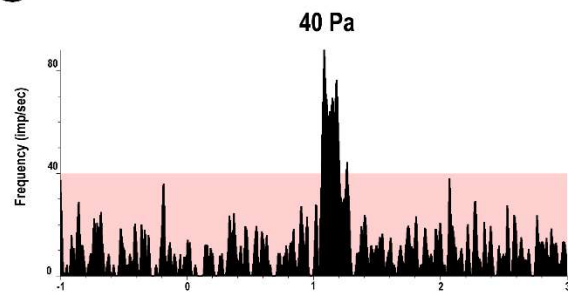
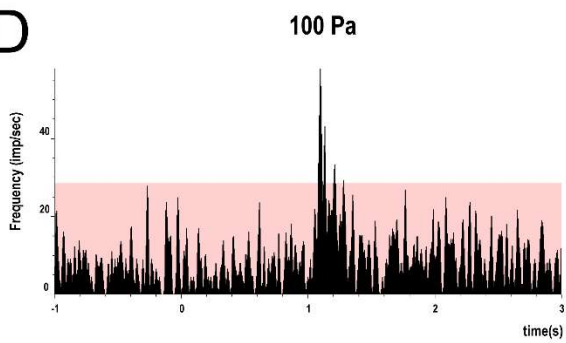
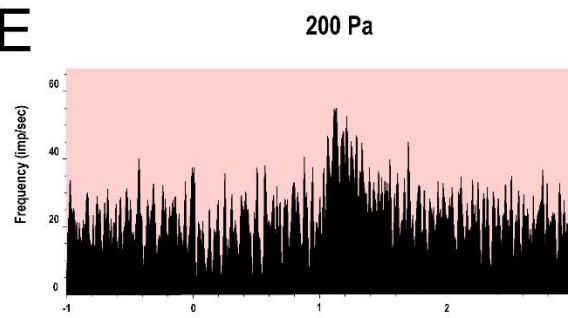
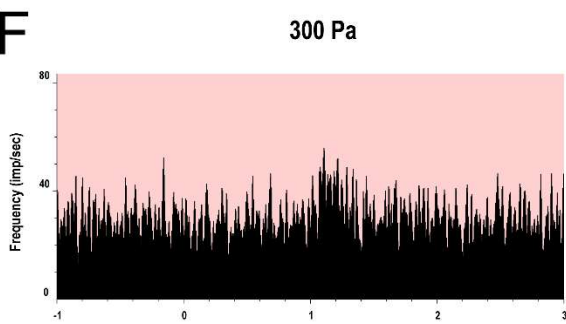
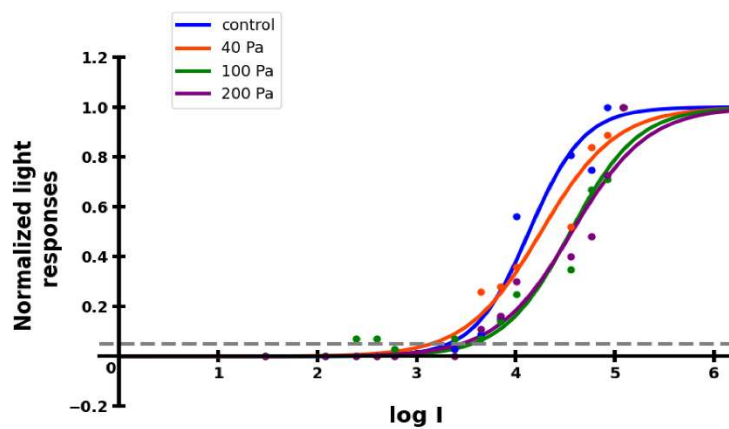
B**C****D****E****F****G**

Figure 3.3.9 Mechanical pressure on α RGCs.

(A) Illustration of the mechanical pressure system.

(B-F) Peristimulus time histograms (PSTHs) of light-evoked responses of OFF α RGCs under different pressures (0, 40, 100, 200, and 300 Pa).

(C) Fitted normalized response-intensity curves for the same α RGC under different pressures (0, 40, 100, and 200 Pa).

3.3.10 α RGC amplitude reduction without reversal potential changes under pressure: three-group analysis of WT mice before and after micropipette compression, and 4-week SOH mice

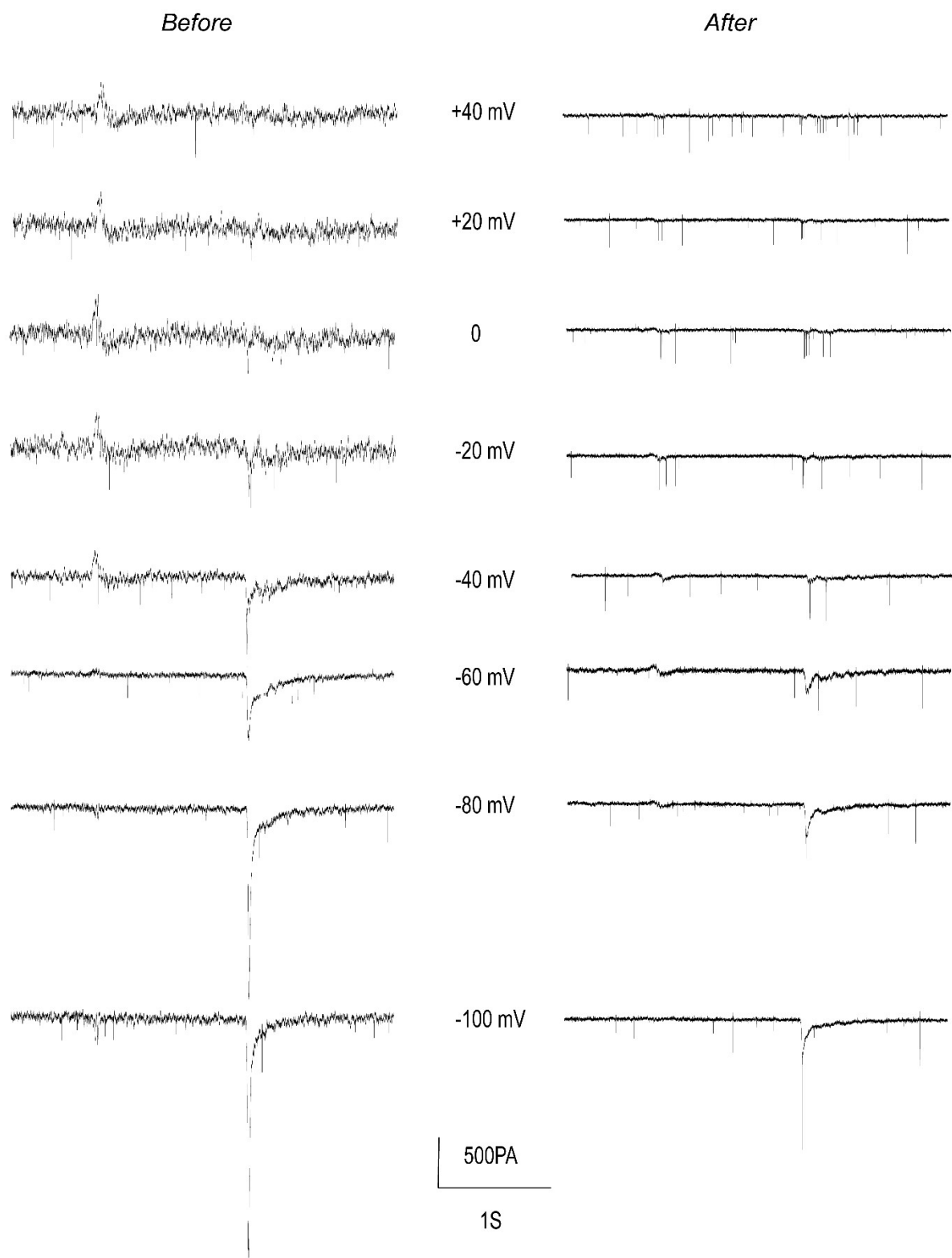
The light-evoked currents of the same α RGC before and after mechanical pressure of WT mice, as well as the α RGCs of 4-week SOH mice, were recorded using the whole-cell technique at a light intensity of 1.2×10^5 Rh*/rod/sec under different holding potentials (-100, -80, -60, -40, -20, 0, 20, and 40 mV) in **Fig. 3.3.10A**.

There are two light-evoked current components exhibited by ON α RGCs at the light onset, referred to as the early component and the late component. The early component is presumed to be driven by BCs, whereas the late component is hypothesized to be mediated by amacrine cells. The light-evoked current–voltage (I–V) relationships of ON α RGCs are represented using line graphs, showing a positive slope (**Fig. 3.3.10 B, C**). In the light-evoked currents of the same α RGCs before and after mechanical pressure in mice, as well as in the α RGCs of 4-week SOH mice, the reversal

potentials of the early components and the late components in all three groups are almost within the same range, corresponding respectively to the equilibrium potential of a cation channel and the equilibrium potential of a chloride channel. Compared to the ON α RGCs before mechanical pressure, the amplitudes of the light-evoked currents in the ON α RGCs after mechanical pressure and in the 4-week silicone oil-treated α RGCs are decreased across different potentials.

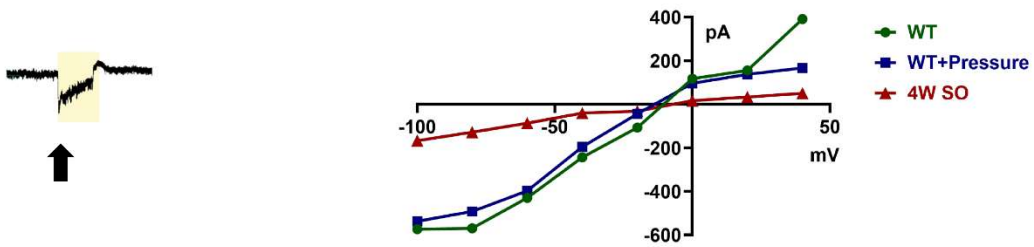
OFF α RGCs exhibit two distinct light responses: one at light onset and another at light offset. The ON response, during the change in potential, does not exhibit a reversal phenomenon due to the regulation of two opponent conductances mediated by HBCs and ACs. In contrast, the OFF response, driven by signals from bipolar cells, reverses near the equilibrium potential of a cation channel. The light-evoked current–voltage (I–V) relationships of ON α RGCs are represented using line graphs, showing a positive slope (**Fig. 3.3.10 D, E**). The reversal potential of the OFF response in OFF α RGCs, both in the mechanical pressure group and the 4-week SOH group, remains close to the equilibrium potential of a cation channel. Compared to OFF α RGCs before mechanical pressure, the amplitude of light-evoked currents in OFF α RGCs subjected to mechanical pressure and those in the 4-week SOH group show a reduction across various potentials.

A



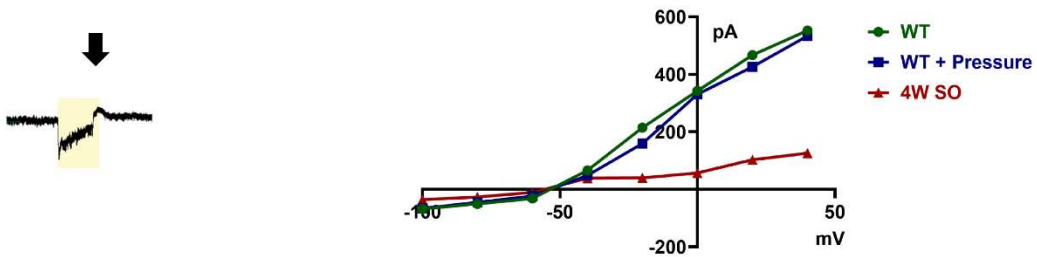
B

Early light response of ON α RGCs



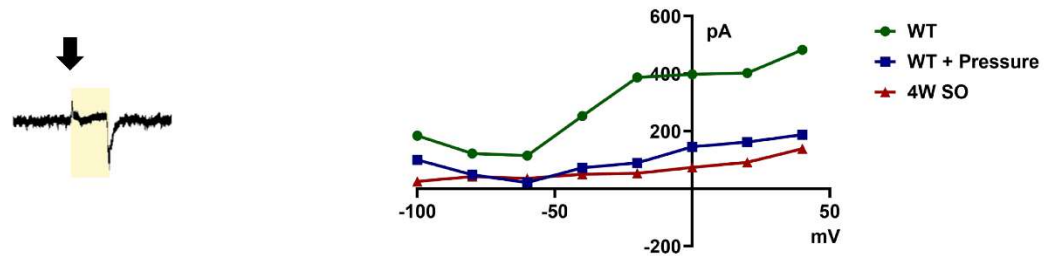
C

Late light response of ON α RGCs



D

ON response of OFF α RGCs



E

OFF response of OFF α RGCs

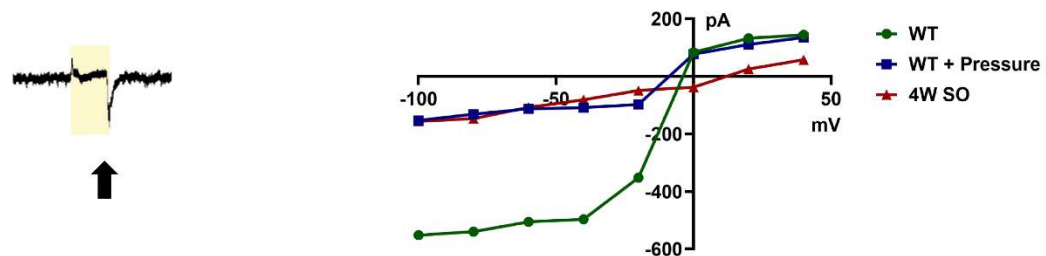


Figure 3.3.10 Light-evoked currents of α RGCs under different holding potentials in control, acute mechanical pressure, and 4-week SOH groups.

(A) Representative light-evoked current of OFF α RGCs at various potentials before and after micropipette compression.

(B) Line graphs of the light-evoked current–voltage (I–V) relationships for the early and late components of ON α RGCs, showing the light-evoked currents in the same ON α RGCs before and after mechanical pressure in mice, as well as in the ON α RGCs of mice treated with 4-week SOH.

(C) Line graphs of the light-evoked current–voltage (I–V) relationships for the ON responses and OFF responses of OFF α RGCs, showing the light-evoked currents in the same OFF α RGCs before and after mechanical pressure in mice, as well as in the OFF α RGCs of mice treated with 4-week SOH.

3.4 Discussion

3.4.1 Retinal ganglion cell loss in two ocular hypertension mouse models

The study utilized two distinct methodologies to induce ocular hypertension in mouse models: silicone oil injection into the anterior chamber to establish sustained IOP elevation, and acute IOP elevation maintained exclusively during the designated 50-minute experimental protocol. α RGCs served as the primary investigational target due to their functional significance, distinctive morphological characteristics, and uniform distribution pattern throughout the retina.

Following anterior chamber silicone oil injection, we observed a significant reduction in α RGC density at the 4-week timepoint, which corroborates findings reported by Zhao et al. regarding α RGC loss in similar experimental paradigms (Zhao et al., 2023). In the acute IOP elevation model, α RGC survival demonstrated a progressive decline at 2-week and 4-week post-intervention intervals. Notably, this loss pattern appeared non-linear, with an apparent deceleration in the rate of α RGC degeneration during later timepoints, potentially attributable to inherent α RGC resilience mechanisms. This observation seems to align with single-cell profiling studies that have categorized α RGC OFF-S and α RGC ON-S subtypes as resilient populations, while α RGC OFF-T subtypes are classified among susceptible RGC populations (Tran et al., 2019). Previous investigations have documented variations in RGC loss kinetics influenced by experimental duration, IOP magnitude, and specie (Leung et al., 2009; Sellés-Navarro et al., 1996; Xuan et al., 2024). Our findings contribute additional parametric data to this field. Notably, at the 4-week timepoint, α RGC survival in the silicone oil group was superior to that observed in the acute IOP

elevation group, suggesting that IOP magnitude may exert a more pronounced influence on neuronal survival than exposure duration, although the underlying mechanisms remain elusive.

3.4.2 Compromised retinal visual function in AOH and SOH mice model

ERG analysis of the SOH group revealed potential functional impairment in both RGCs and ACs. Given the critical role of AII ACs in visual processing pathways, quantitative analysis was extended beyond α RGCs to include AII amacrine cells. No significant reduction in AII ACs density was observed, which may reflect the characteristic pattern in early glaucoma whereby functional deficits precede cellular loss, a feature that could facilitate early glaucomatous diagnosis. Previous studies utilizing microbead occlusion and laser/cautery coagulation methods in experimental glaucoma mouse models have reported reduced sensitivity in AII amacrine cells, ON α RGCs, and sustained OFF α RGCs (Pang et al., 2015). However, in our experimental paradigm, patch-clamp recordings from individual cells demonstrated decreased light response thresholds across all four α RGC subtypes.

In the AOH model, optomotor response recordings demonstrated a mild decline in visual contrast sensitivity at 2 weeks, progressing to significant impairment at 4 weeks. In comparison, α RGC density exhibited a significant reduction as early as 2 weeks post-intervention, suggesting a transient functional reserve among surviving cells. Additionally, whole-cell patch-clamp recordings revealed increased light response thresholds for both EPSCs and IPSCs in α RGCs, indicating compromised excitatory and inhibitory synaptic inputs. These findings indicate that synaptic dysfunction represents an early pathological event that may drive subsequent α RGC

degeneration and visual impairment. However, the underlying mechanisms driving these synaptic alterations remain to be elucidated.

3.4.3 Mechanical pressure on α RGC

We subsequently applied graded mechanical pressure directly to the α RGC surface. Results demonstrated that increasing pressure progressively elevated light sensitivity thresholds while simultaneously increasing the number of light-induced spikes. This phenomenon may reflect injury-related noise amplification caused by mechanical stress, or alternatively, activation of surface Piezo channels triggering additional action potential. Piezo 1 channel is a mechanically activated, cation-selective channel responsive to mechanical stimuli. At the retinal level, Piezo1 channels are exclusively expressed in the GCL, while Piezo2 channels are present in both retinal ganglion cells and the optic nerve head. Previous studies have demonstrated upregulated Piezo channel expression in glaucoma animal models, potentially contributing to RGC dysfunction under elevated IOP conditions (Choi et al., 2015; Morozumi et al., 2020). GsMTx4, a Piezo channel antagonist, may represent a promising neuroprotective therapeutic strategy (Pang, 2024).

To further elucidate the mechanisms underlying pressure-induced RGC dysfunction, we conducted direct mechanical stimulation experiments on individual RGCs from WT mice before and after micropipette compression application, while holding cells at different membrane potentials. Our findings revealed that the reversal potential remained largely unchanged following mechanical pressure application, suggesting that the fundamental ionic composition and driving forces of synaptic currents were preserved (Pang et al., 2003). However, we observed a notable reduction in postsynaptic current amplitude, indicating compromised synaptic transmission efficacy under

mechanical stress. This finding is consistent with the hypothesis that mechanical pressure primarily affects presynaptic function.

In parallel experiments using α RGCs from 4-week SOH mice, we observed a similar pattern of unchanged reversal potential but significantly reduced postsynaptic current amplitude. This concordance between mechanical pressure and SOH models strengthens the notion that elevated pressure, regardless of duration, primarily compromises synaptic transmission efficiency rather than altering the fundamental biophysical properties of synaptic responses. The more pronounced amplitude reduction in the chronic model suggests that prolonged pressure exposure may exacerbate synaptic dysfunction through cumulative damage mechanisms.

These electrophysiological findings provide direct evidence for pressure-induced synaptic impairment and support the emerging concept that synaptic dysfunction precedes RGC death in glaucomatous pathology.

3.5 Conclusion

This study provides compelling evidence that the retina serves as a primary target of elevated intraocular pressure-induced injury in glaucomatous pathophysiology. Through our comprehensive experimental approach utilizing both SOH and AOH in vivo models, we demonstrated that sustained IOP elevation precipitates profound synaptic dysfunction within retinal neural networks. This dysfunction is characterized predominantly by aberrant amplification of light-evoked synaptic responses, indicating fundamental disruptions in normal synaptic transmission and visual signal processing mechanisms.

Our complementary in vitro investigations further revealed that direct mechanical stress applied to retinal ganglion cells significantly compromises the precision of visual signal encoding at the retinal level. These findings suggest that retinal neuronal dysfunction represents a critical pathophysiological mechanism underlying glaucomatous vision loss.

In conclusion, such distortions in foundational visual processing likely cascade through hierarchical visual pathways, ultimately manifesting as the complex pattern of visual field defects and cortical signal acquisition abnormalities characteristic of advanced glaucomatous disease.

Chapter 4 Overall Conclusion

RGCs are the final output neurons in the vertebrate retina that project to the brain, thus occupying an indispensable position in the visual pathway. Given that RGC damage is irreversible, neuroprotective approaches become paramount.

The thesis investigation demonstrates the mechanisms by which two primary pathological conditions--chemical alkali injury to the cornea and increased intraocular pressure--impair RGCs via separate but interconnected pathways.

In corneal alkaline exposure, RGC apoptosis exhibited a characteristic spatial-temporal pattern, progressing from the far-peripheral region toward the central retinal area. However, this apoptosis was not directly attributed to pH alterations, indicating that alternative molecular pathways mediate the observed neuronal damage. TNF- α activation emerges as a potential key mechanism underlying this process, likely triggering downstream inflammatory cascades that contribute to sustained retinal degeneration.

Cx36 gap junctions play a crucial role in secondary cell death throughout the damaged retinal tissue, effectively accelerate and amplify RGC apoptosis beyond the primary injury zone. This gap junction-mediated mechanism represents a novel therapeutic target, as it suggests that intercellular communication pathways can be modulated to limit the extent of retinal damage.

Significantly, blocking Cx36 through either genetic knockout or pharmacological intervention during the early stages of injury could effectively protect RGCs from the secondary cell death. MFA, functioning as a gap junction blocker, showed protective neuronal effects through inhibition of this secondary apoptotic pathway, suggesting its potential as a therapeutic neuroprotective drug for post-alkali burn treatment.

Experimental IOP elevation, whether acute or sustained, consistently resulted in retinal damage characterized by RGC loss, emphasizing the critical importance of understanding elevated IOP's deleterious effects on retinal function.

Early-stage behavioral deficits exhibited a complex masking phenomenon mediated by compensatory mechanisms within the visual system. These adaptive responses are likely contributed by neural plasticity at multiple levels of the visual pathway, which leads to temporary preservation of apparent visual function despite underlying structural damage. Damage to fundamental synaptic function and compromised cell survival could be reliably detected even when behavioral measures remained within normal ranges. These findings provide crucial new insights for developing more sensitive early diagnostic approaches.

Direct pressure manipulation experiments provide a mechanistic link between elevated IOP and visual dysfunction by revealing immediate reductions in light sensitivity and synaptic transmission, potentially due to spurious spiking and baseline fluctuations that mask genuine visual signals. These responses suggest that mechanical pressure directly compromises RGC function likely through membrane destabilization, ion channel dysfunction, and altered neurotransmitter release. This mechanical pressure-induced dysfunction provides a foundational understanding of how sustained IOP elevation in glaucoma progressively degrades visual processing, offering valuable insights into the biophysical mechanisms underlying pressure-induced retinal degeneration and informing the development of targeted neuroprotective strategies.

Overall, the retina represents a delicate yet highly sophisticated neural structure, making it crucial to investigate retinal damage under various pathological conditions and develop protective strategies. RGC signaling characteristics reveal initial pathological alterations and offer cellular-level mechanisms for developing early-intervention approaches against ocular disorders.

Reference

- Agar, A., Yip, S. S., Hill, M. A., & Coroneo, M. T. (2000). Pressure related apoptosis in neuronal cell lines. *J Neurosci Res*, 60(4), 495–503. [https://doi.org/10.1002/\(sici\)1097-4547\(20000515\)60:4<495::Aid-jnr8>3.0.Co;2-s](https://doi.org/10.1002/(sici)1097-4547(20000515)60:4<495::Aid-jnr8>3.0.Co;2-s)
- Ahmed, F. A., Chaudhary, P., & Sharma, S. C. (2001). Effects of increased intraocular pressure on rat retinal ganglion cells. *Int J Dev Neurosci*, 19(2), 209–218. [https://doi.org/10.1016/s0736-5748\(00\)00073-3](https://doi.org/10.1016/s0736-5748(00)00073-3)
- Akopian, A., Atlasz, T., Pan, F., Wong, S., Zhang, Y., Völgyi, B., Paul, D. L., & Bloomfield, S. A. (2014). Gap junction-mediated death of retinal neurons is connexin and insult specific: a potential target for neuroprotection. *J Neurosci*, 34(32), 10582–10591. <https://doi.org/10.1523/jneurosci.1912-14.2014>
- Akopian, A., Kumar, S., Ramakrishnan, H., Roy, K., Viswanathan, S., & Bloomfield, S. A. (2017). Targeting neuronal gap junctions in mouse retina offers neuroprotection in glaucoma. *J Clin Invest*, 127(7), 2647–2661. <https://doi.org/10.1172/jci91948>
- Applebury, M. L., Antoch, M. P., Baxter, L. C., Chun, L. L. Y., Falk, J. D., Farhangfar, F., Kage, K., Krzystolik, M. G., Lyass, L. A., & Robbins, J. T. (2000). The Murine Cone Photoreceptor: A Single Cone Type Expresses Both S and M Opsins with Retinal Spatial Patterning. *Neuron*, 27(3), 513–523. [https://doi.org/10.1016/S0896-6273\(00\)00062-3](https://doi.org/10.1016/S0896-6273(00)00062-3)
- Asrani, S. G., McGlumphy, E. J., Al-Aswad, L. A., Chaya, C. J., Lin, S., Musch, D. C., Pitha, I., Robin, A. L., Wiostko, B., & Johnson, T. V. (2024). The relationship between intraocular pressure and glaucoma: An evolving concept. *Prog Retin Eye Res*, 103, 101303. <https://doi.org/10.1016/j.preteyeres.2024.101303>

- Attwell, D., Wilson, M., & Wu, S. M. (1984). A quantitative analysis of interactions between photoreceptors in the salamander (*Ambystoma*) retina. *J Physiol*, 352, 703–737.
<https://doi.org/10.1113/jphysiol.1984.sp015318>
- Baden, T., Berens, P., Bethge, M., & Euler, T. (2013). Spikes in mammalian bipolar cells support temporal layering of the inner retina. *Curr Biol*, 23(1), 48–52.
<https://doi.org/10.1016/j.cub.2012.11.006>
- Baden, T., Berens, P., Franke, K., Román Rosón, M., Bethge, M., & Euler, T. (2016). The functional diversity of retinal ganglion cells in the mouse. *Nature*, 529(7586), 345–350.
<https://doi.org/10.1038/nature16468>
- Bae, J. A., Mu, S., Kim, J. S., Turner, N. L., Tartavull, I., Kemnitz, N., Jordan, C. S., Norton, A. D., Silversmith, W. M., Prentki, R., Sorek, M., David, C., Jones, D. L., Bland, D., Sterling, A. L. R., Park, J., Briggman, K. L., & Seung, H. S. (2018). Digital Museum of Retinal Ganglion Cells with Dense Anatomy and Physiology. *Cell*, 173(5), 1293–1306.e1219. <https://doi.org/10.1016/j.cell.2018.04.040>
- Ballen, P. H. (1964). TREATMENT OF CHEMICAL BURNS OF THE EYE. *Eye Ear Nose Throat Mon*, 43, 57–61.
- Ballesta, J., Terenghi, G., Thibault, J., & Polak, J. M. (1984). Putative dopamine-containing cells in the retina of seven species demonstrated by tyrosine hydroxylase immunocytochemistry. *Neuroscience*, 12(4), 1147–1156. [https://doi.org/10.1016/0306-4522\(84\)90009-5](https://doi.org/10.1016/0306-4522(84)90009-5)
- Banerjee, S., Wang, Q., Zhao, F., Tang, G., So, C., Tse, D., To, C. H., Feng, Y., Zhou, X., & Pan, F. (2020). Increased Connexin36 Phosphorylation in AII Amacrine Cell Coupling of the

- Mouse Myopic Retina. *Front Cell Neurosci*, 14, 124.
<https://doi.org/10.3389/fncel.2020.00124>
- Baradaran-Rafii, A., Eslani, M., Haq, Z., Shirzadeh, E., Huvard, M. J., & Djalilian, A. R. (2017). Current and Upcoming Therapies for Ocular Surface Chemical Injuries. *Ocul Surf*, 15(1), 48–64. <https://doi.org/10.1016/j.jtos.2016.09.002>
- Barlow, H. B., Hill, R. M., & Levick, W. R. (1964). RETINAL GANGLION CELLS RESPONDING SELECTIVELY TO DIRECTION AND SPEED OF IMAGE MOTION IN THE RABBIT. *J Physiol*, 173(3), 377–407.
<https://doi.org/10.1113/jphysiol.1964.sp007463>
- Barlow, H. B., & Levick, W. R. (1965). The mechanism of directionally selective units in rabbit's retina. *J Physiol*, 178(3), 477–504. <https://doi.org/10.1113/jphysiol.1965.sp007638>
- Barnard, A. R., Hattar, S., Hankins, M. W., & Lucas, R. J. (2006). Melanopsin regulates visual processing in the mouse retina. *Curr Biol*, 16(4), 389–395.
<https://doi.org/10.1016/j.cub.2005.12.045>
- Bedner, P., Steinhäuser, C., & Theis, M. (2012). Functional redundancy and compensation among members of gap junction protein families? *Biochim Biophys Acta*, 1818(8), 1971–1984. <https://doi.org/10.1016/j.bbamem.2011.10.016>
- Besharse, J. C., & McMahon, D. G. (2016). The Retina and Other Light-sensitive Ocular Clocks. *J Biol Rhythms*, 31(3), 223–243. <https://doi.org/10.1177/0748730416642657>
- Beyer, E. C., & Berthoud, V. M. (2018). Gap junction gene and protein families: Connexins, innexins, and pannexins. *Biochim Biophys Acta Biomembr*, 1860(1), 5–8.
<https://doi.org/10.1016/j.bbamem.2017.05.016>

- Beyer, E. C., Paul, D. L., & Goodenough, D. A. (1987). Connexin43: a protein from rat heart homologous to a gap junction protein from liver. *J Cell Biol*, 105(6 Pt 1), 2621–2629. <https://doi.org/10.1083/jcb.105.6.2621>
- Bloomfield, S. A., & Völgyi, B. (2004). Function and plasticity of homologous coupling between AII amacrine cells. *Vision Res*, 44(28), 3297–3306. <https://doi.org/10.1016/j.visres.2004.07.012>
- Bloomfield, S. A., & Völgyi, B. (2009). The diverse functional roles and regulation of neuronal gap junctions in the retina. *Nat Rev Neurosci*, 10(7), 495–506. <https://doi.org/10.1038/nrn2636>
- Bloomfield, S. A., Xin, D., & Osborne, T. (1997). Light-induced modulation of coupling between AII amacrine cells in the rabbit retina. *Vis Neurosci*, 14(3), 565–576. <https://doi.org/10.1017/s0952523800012220>
- Borghuis, B. G., Marvin, J. S., Looger, L. L., & Demb, J. B. (2013). Two-photon imaging of nonlinear glutamate release dynamics at bipolar cell synapses in the mouse retina. *J Neurosci*, 33(27), 10972–10985. <https://doi.org/10.1523/jneurosci.1241-13.2013>
- Boycott, B. B., & Wässle, H. (1974). The morphological types of ganglion cells of the domestic cat's retina. *J Physiol*, 240(2), 397–419. <https://doi.org/10.1113/jphysiol.1974.sp010616>
- Brainard, G. C., & Morgan, W. W. (1987). Light-induced stimulation of retinal dopamine: a dose-response relationship. *Brain Res*, 424(1), 199–203. [https://doi.org/10.1016/0006-8993\(87\)91211-x](https://doi.org/10.1016/0006-8993(87)91211-x)
- Brandstätter, J. H., Koulen, P., & Wässle, H. (1998). Diversity of glutamate receptors in the mammalian retina. *Vision Res*, 38(10), 1385–1397. [https://doi.org/10.1016/s0042-6989\(97\)00176-4](https://doi.org/10.1016/s0042-6989(97)00176-4)

- Breuninger, T., Puller, C., Haverkamp, S., & Euler, T. (2011). Chromatic bipolar cell pathways in the mouse retina. *J Neurosci*, 31(17), 6504–6517. <https://doi.org/10.1523/jneurosci.0616-11.2011>
- Brivanlou, I. H., Warland, D. K., & Meister, M. (1998). Mechanisms of concerted firing among retinal ganglion cells. *Neuron*, 20(3), 527–539. [https://doi.org/10.1016/s0896-6273\(00\)80992-7](https://doi.org/10.1016/s0896-6273(00)80992-7)
- Brunet, A. A., Harvey, A. R., & Carvalho, L. S. (2022). Primary and Secondary Cone Cell Death Mechanisms in Inherited Retinal Diseases and Potential Treatment Options. *Int J Mol Sci*, 23(2). <https://doi.org/10.3390/ijms23020726>
- Cade, F., Grosskreutz, C. L., Tauber, A., & Dohlman, C. H. (2011). Glaucoma in eyes with severe chemical burn, before and after keratoprosthesis. *Cornea*, 30(12), 1322–1327. <https://doi.org/10.1097/ICO.0b013e31821eead6>
- Cade, F., Paschalis, E. I., Regatieri, C. V., Vavvas, D. G., Dana, R., & Dohlman, C. H. (2014). Alkali burn to the eye: protection using TNF- α inhibition. *Cornea*, 33(4), 382–389. <https://doi.org/10.1097/ico.0000000000000071>
- Cadetti, L., & Thoreson, W. B. (2006). Feedback effects of horizontal cell membrane potential on cone calcium currents studied with simultaneous recordings. *J Neurophysiol*, 95(3), 1992–1995. <https://doi.org/10.1152/jn.01042.2005>
- Chalupa, L. M., & Günhan, E. (2004). Development of On and Off retinal pathways and retinogeniculate projections. *Prog Retin Eye Res*, 23(1), 31–51. <https://doi.org/10.1016/j.preteyeres.2003.10.001>

- Chaya, T., Matsumoto, A., Sugita, Y., Watanabe, S., Kuwahara, R., Tachibana, M., & Furukawa, T. (2017). Versatile functional roles of horizontal cells in the retinal circuit. *Sci Rep*, 7(1), 5540. <https://doi.org/10.1038/s41598-017-05543-2>
- Chesler, M. (2003). Regulation and modulation of pH in the brain. *Physiol Rev*, 83(4), 1183–1221. <https://doi.org/10.1152/physrev.00010.2003>
- Choi, H. J., Sun, D., & Jakobs, T. C. (2015). Astrocytes in the optic nerve head express putative mechanosensitive channels. *Mol Vis*, 21, 749–766.
- Choudhury, S., Liu, Y., Clark, A. F., & Pang, I. H. (2015). Caspase-7: a critical mediator of optic nerve injury-induced retinal ganglion cell death. *Mol Neurodegener*, 10, 40. <https://doi.org/10.1186/s13024-015-0039-2>
- Church, J., & Baimbridge, K. G. (1991). Exposure to high-pH medium increases the incidence and extent of dye coupling between rat hippocampal CA1 pyramidal neurons in vitro. *J Neurosci*, 11(10), 3289–3295. <https://doi.org/10.1523/jneurosci.11-10-03289.1991>
- Cleland, B. G., & Levick, W. R. (1974). Brisk and sluggish concentrically organized ganglion cells in the cat's retina. *J Physiol*, 240(2), 421–456. <https://doi.org/10.1113/jphysiol.1974.sp010617>
- Cleland, B. G., Levick, W. R., & Wässle, H. (1975). Physiological identification of a morphological class of cat retinal ganglion cells. *J Physiol*, 248(1), 151–171. <https://doi.org/10.1113/jphysiol.1975.sp010967>
- Cohen, A. I., Todd, R. D., Harmon, S., & O'Malley, K. L. (1992). Photoreceptors of mouse retinas possess D4 receptors coupled to adenylate cyclase. *Proc Natl Acad Sci U S A*, 89(24), 12093–12097. <https://doi.org/10.1073/pnas.89.24.12093>

- Connaughton, V. P., Graham, D., & Nelson, R. (2004). Identification and morphological classification of horizontal, bipolar, and amacrine cells within the zebrafish retina. *J Comp Neurol*, 477(4), 371–385. <https://doi.org/10.1002/cne.20261>
- Connors, B. W., & Long, M. A. (2004). Electrical synapses in the mammalian brain. *Annu Rev Neurosci*, 27, 393–418. <https://doi.org/10.1146/annurev.neuro.26.041002.131128>
- Crowston, J. G., Kong, Y. X., Trounce, I. A., Dang, T. M., Fahy, E. T., Bui, B. V., Morrison, J. C., & Chrysostomou, V. (2015). An acute intraocular pressure challenge to assess retinal ganglion cell injury and recovery in the mouse. *Exp Eye Res*, 141, 3–8. <https://doi.org/10.1016/j.exer.2015.03.006>
- D'Souza, S., & Lang, R. A. (2020). Retinal ganglion cell interactions shape the developing mammalian visual system. *Development*, 147(23). <https://doi.org/10.1242/dev.196535>
- Dacheux, R. F., & Raviola, E. (1986). The rod pathway in the rabbit retina: a depolarizing bipolar and amacrine cell. *J Neurosci*, 6(2), 331–345. <https://doi.org/10.1523/jneurosci.06-02-00331.1986>
- Das, S., Lin, D., Jena, S., Shi, A., Battina, S., Hua, D. H., Allbaugh, R., & Takemoto, D. J. (2008). Protection of retinal cells from ischemia by a novel gap junction inhibitor. *Biochem Biophys Res Commun*, 373(4), 504–508. <https://doi.org/10.1016/j.bbrc.2008.06.069>
- de Vries, S. E., Baccus, S. A., & Meister, M. (2011). The projective field of a retinal amacrine cell. *J Neurosci*, 31(23), 8595–8604. <https://doi.org/10.1523/jneurosci.5662-10.2011>
- Deans, M. R., Gibson, J. R., Sellitto, C., Connors, B. W., & Paul, D. L. (2001). Synchronous activity of inhibitory networks in neocortex requires electrical synapses containing connexin36. *Neuron*, 31(3), 477–485. [https://doi.org/10.1016/s0896-6273\(01\)00373-7](https://doi.org/10.1016/s0896-6273(01)00373-7)

- Deans, M. R., Volgyi, B., Goodenough, D. A., Bloomfield, S. A., & Paul, D. L. (2002). Connexin36 is essential for transmission of rod-mediated visual signals in the mammalian retina. *Neuron*, 36(4), 703–712. [https://doi.org/10.1016/s0896-6273\(02\)01046-2](https://doi.org/10.1016/s0896-6273(02)01046-2)
- Derouiche, A., & Asan, E. (1999). The dopamine D2 receptor subfamily in rat retina: ultrastructural immunogold and in situ hybridization studies. *Eur J Neurosci*, 11(4), 1391–1402. <https://doi.org/10.1046/j.1460-9568.1999.00557.x>
- DeVries, S. H. (2000). Bipolar cells use kainate and AMPA receptors to filter visual information into separate channels. *Neuron*, 28(3), 847–856. [https://doi.org/10.1016/s0896-6273\(00\)00158-6](https://doi.org/10.1016/s0896-6273(00)00158-6)
- DeVries, S. H., Li, W., & Saszik, S. (2006). Parallel processing in two transmitter microenvironments at the cone photoreceptor synapse. *Neuron*, 50(5), 735–748. <https://doi.org/10.1016/j.neuron.2006.04.034>
- DeVries, S. H., Qi, X., Smith, R., Makous, W., & Sterling, P. (2002). Electrical coupling between mammalian cones. *Curr Biol*, 12(22), 1900–1907. [https://doi.org/10.1016/s0960-9822\(02\)01261-7](https://doi.org/10.1016/s0960-9822(02)01261-7)
- Diamond, J. S. (2017). Inhibitory Interneurons in the Retina: Types, Circuitry, and Function. *Annu Rev Vis Sci*, 3, 1–24. <https://doi.org/10.1146/annurev-vision-102016-061345>
- Doucette, L. P., Rasnitsyn, A., Seifi, M., & Walter, M. A. (2015). The interactions of genes, age, and environment in glaucoma pathogenesis. *Surv Ophthalmol*, 60(4), 310–326. <https://doi.org/10.1016/j.survophthal.2015.01.004>

- Downs, J. C., Roberts, M. D., & Burgoyne, C. F. (2008). Mechanical environment of the optic nerve head in glaucoma. *Optom Vis Sci*, 85(6), 425–435.
<https://doi.org/10.1097/OPX.0b013e31817841cb>
- Doyle, S. E., McIvor, W. E., & Menaker, M. (2002). Circadian rhythmicity in dopamine content of mammalian retina: role of the photoreceptors. *J Neurochem*, 83(1), 211–219.
<https://doi.org/10.1046/j.1471-4159.2002.01149.x>
- Dua, H. S., King, A. J., & Joseph, A. (2001). A new classification of ocular surface burns. *Br J Ophthalmol*, 85(11), 1379–1383. <https://doi.org/10.1136/bjo.85.11.1379>
- Duan, X., Qiao, M., Bei, F., Kim, I. J., He, Z., & Sanes, J. R. (2015). Subtype-specific regeneration of retinal ganglion cells following axotomy: effects of osteopontin and mTOR signaling. *Neuron*, 85(6), 1244–1256.
<https://doi.org/10.1016/j.neuron.2015.02.017>
- Eggers, E. D., & Lukasiewicz, P. D. (2006). GABA(A), GABA(C) and glycine receptor-mediated inhibition differentially affects light-evoked signalling from mouse retinal rod bipolar cells. *J Physiol*, 572(Pt 1), 215–225. <https://doi.org/10.1113/jphysiol.2005.103648>
- Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxicol Pathol*, 35(4), 495–516. <https://doi.org/10.1080/01926230701320337>
- Euler, T., Haverkamp, S., Schubert, T., & Baden, T. (2014). Retinal bipolar cells: elementary building blocks of vision. *Nat Rev Neurosci*, 15(8), 507–519.
<https://doi.org/10.1038/nrn3783>
- Famiglietti, E. V., Jr., & Kolb, H. (1976). Structural basis for ON-and OFF-center responses in retinal ganglion cells. *Science*, 194(4261), 193–195.
<https://doi.org/10.1126/science.959847>

- Famiglietti, E. V., & Kolb, H. (1975). A bistratified amacrine cell and synaptic circuitry in the inner plexiform layer of the retina. *Brain Research*, 84(2), 293–300.
[https://doi.org/https://doi.org/10.1016/0006-8993\(75\)90983-X](https://doi.org/https://doi.org/10.1016/0006-8993(75)90983-X)
- Farshi, P., Fyk-Kolodziej, B., Krolewski, D. M., Walker, P. D., & Ichinose, T. (2016). Dopamine D1 receptor expression is bipolar cell type-specific in the mouse retina. *J Comp Neurol*, 524(10), 2059–2079. <https://doi.org/10.1002/cne.23932>
- Feigenspan, A., Teubner, B., Willecke, K., & Weiler, R. (2001). Expression of neuronal connexin36 in AII amacrine cells of the mammalian retina. *J Neurosci*, 21(1), 230–239.
<https://doi.org/10.1523/jneurosci.21-01-00230.2001>
- Ferrari, G., Bignami, F., Giacomini, C., Franchini, S., & Rama, P. (2013). Safety and efficacy of topical infliximab in a mouse model of ocular surface scarring. *Invest Ophthalmol Vis Sci*, 54(3), 1680–1688. <https://doi.org/10.1167/iovs.12-10782>
- Flood, M. D., & Eggers, E. D. (2021). Dopamine D1 and D4 receptors contribute to light adaptation in ON-sustained retinal ganglion cells. *J Neurophysiol*, 126(6), 2039–2052.
<https://doi.org/10.1152/jn.00218.2021>
- Flores-Herr, N., Protti, D. A., & Wässle, H. (2001). Synaptic currents generating the inhibitory surround of ganglion cells in the mammalian retina. *J Neurosci*, 21(13), 4852–4863.
<https://doi.org/10.1523/jneurosci.21-13-04852.2001>
- Frankfort, B. J., Khan, A. K., Tse, D. Y., Chung, I., Pang, J. J., Yang, Z., Gross, R. L., & Wu, S. M. (2013). Elevated intraocular pressure causes inner retinal dysfunction before cell loss in a mouse model of experimental glaucoma. *Invest Ophthalmol Vis Sci*, 54(1), 762–770.
<https://doi.org/10.1167/iovs.12-10581>

- Fu, Q. L., Li, X., Shi, J., Xu, G., Wen, W., Lee, D. H., & So, K. F. (2009). Synaptic degeneration of retinal ganglion cells in a rat ocular hypertension glaucoma model. *Cell Mol Neurobiol*, 29(4), 575–581. <https://doi.org/10.1007/s10571-009-9349-7>
- Fu, Y., & Yau, K. W. (2007). Phototransduction in mouse rods and cones. *Pflugers Arch*, 454(5), 805–819. <https://doi.org/10.1007/s00424-006-0194-y>
- Fuchshofer, R., & Tamm, E. R. (2012). The role of TGF- β in the pathogenesis of primary open-angle glaucoma. *Cell Tissue Res*, 347(1), 279–290. <https://doi.org/10.1007/s00441-011-1274-7>
- Gallego-Ortega, A., Norte-Muñoz, M., Di Pierdomenico, J., Avilés-Trigueros, M., de la Villa, P., Valiente-Soriano, F. J., & Vidal-Sanz, M. (2022). Alpha retinal ganglion cells in pigmented mice retina: number and distribution. *Front Neuroanat*, 16, 1054849. <https://doi.org/10.3389/fnana.2022.1054849>
- Godley, B. F., & Wurtman, R. J. (1988). Release of endogenous dopamine from the superfused rabbit retina in vitro: effect of light stimulation. *Brain Res*, 452(1-2), 393–395. [https://doi.org/10.1016/0006-8993\(88\)90046-7](https://doi.org/10.1016/0006-8993(88)90046-7)
- Goetz, J., Jessen, Z. F., Jacobi, A., Mani, A., Cooler, S., Greer, D., Kadri, S., Segal, J., Shekhar, K., Sanes, J. R., & Schwartz, G. W. (2022). Unified classification of mouse retinal ganglion cells using function, morphology, and gene expression. *Cell Rep*, 40(2), 111040. <https://doi.org/10.1016/j.celrep.2022.111040>
- Goldberg, G. S., Valiunas, V., & Brink, P. R. (2004). Selective permeability of gap junction channels. *Biochim Biophys Acta*, 1662(1-2), 96–101. <https://doi.org/10.1016/j.bbamem.2003.11.022>

- González-Nieto, D., Gómez-Hernández, J. M., Larrosa, B., Gutiérrez, C., Muñoz, M. D., Fasciani, I., O'Brien, J., Zappalà, A., Cicirata, F., & Barrio, L. C. (2008). Regulation of neuronal connexin-36 channels by pH. *Proc Natl Acad Sci U S A*, 105(44), 17169–17174. <https://doi.org/10.1073/pnas.0804189105>
- Grimes, W. N., Baudin, J., Azevedo, A. W., & Rieke, F. (2018). Range, routing and kinetics of rod signaling in primate retina. *Elife*, 7. <https://doi.org/10.7554/eLife.38281>
- Gu, X. J., Liu, X., Chen, Y. Y., Zhao, Y., Xu, M., Han, X. J., Liu, Q. P., Yi, J. L., & Li, J. M. (2016). Involvement of NADPH oxidases in alkali burn-induced corneal injury. *Int J Mol Med*, 38(1), 75–82. <https://doi.org/10.3892/ijmm.2016.2594>
- Güldenagel, M., Söhl, G., Plum, A., Traub, O., Teubner, B., Weiler, R., & Willecke, K. (2000). Expression patterns of connexin genes in mouse retina. *J Comp Neurol*, 425(2), 193–201.
- Guo, L., Moss, S. E., Alexander, R. A., Ali, R. R., Fitzke, F. W., & Cordeiro, M. F. (2005). Retinal ganglion cell apoptosis in glaucoma is related to intraocular pressure and IOP-induced effects on extracellular matrix. *Invest Ophthalmol Vis Sci*, 46(1), 175–182. <https://doi.org/10.1167/iovs.04-0832>
- Hack, I., & Peichl, L. (1999). Horizontal cells of the rabbit retina are non-selectively connected to the cones. *Eur J Neurosci*, 11(7), 2261–2274. <https://doi.org/10.1046/j.1460-9568.1999.00647.x>
- Hayreh, S. S., Pe'er, J., & Zimmerman, M. B. (1999). Morphologic changes in chronic high-pressure experimental glaucoma in rhesus monkeys. *J Glaucoma*, 8(1), 56–71.
- Heijl, A., Leske, M. C., Bengtsson, B., Hyman, L., Bengtsson, B., & Hussein, M. (2002). Reduction of intraocular pressure and glaucoma progression: results from the Early

- Manifest Glaucoma Trial. *Arch Ophthalmol*, 120(10), 1268–1279.
<https://doi.org/10.1001/archopht.120.10.1268>
- Helmstaedter, M., Briggman, K. L., Turaga, S. C., Jain, V., Seung, H. S., & Denk, W. (2013). Connectomic reconstruction of the inner plexiform layer in the mouse retina. *Nature*, 500(7461), 168–174. <https://doi.org/10.1038/nature12346>
- Hidaka, S., Akahori, Y., & Kurosawa, Y. (2004). Dendrodendritic electrical synapses between mammalian retinal ganglion cells. *J Neurosci*, 24(46), 10553–10567.
<https://doi.org/10.1523/jneurosci.3319-04.2004>
- Hombach, S., Janssen-Bienhold, U., Söhl, G., Schubert, T., Büssow, H., Ott, T., Weiler, R., & Willecke, K. (2004). Functional expression of connexin57 in horizontal cells of the mouse retina. *Eur J Neurosci*, 19(10), 2633–2640. <https://doi.org/10.1111/j.0953-816X.2004.03360.x>
- Ibarguren, M., López, D. J., & Escribá, P. V. (2014). The effect of natural and synthetic fatty acids on membrane structure, microdomain organization, cellular functions and human health. *Biochim Biophys Acta*, 1838(6), 1518–1528.
<https://doi.org/10.1016/j.bbamem.2013.12.021>
- Jackson, C. R., Ruan, G. X., Aseem, F., Abey, J., Gamble, K., Stanwood, G., Palmiter, R. D., Iuvone, P. M., & McMahon, D. G. (2012). Retinal dopamine mediates multiple dimensions of light-adapted vision. *J Neurosci*, 32(27), 9359–9368.
<https://doi.org/10.1523/jneurosci.0711-12.2012>
- Jacobs, G. H., Neitz, J., & Deegan, J. F., 2nd. (1991). Retinal receptors in rodents maximally sensitive to ultraviolet light. *Nature*, 353(6345), 655–656.
<https://doi.org/10.1038/353655a0>

- Jan, R., & Chaudhry, G. E. (2019). Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics. *Adv Pharm Bull*, 9(2), 205–218.
<https://doi.org/10.15171/apb.2019.024>
- Jayaram, H., Kolko, M., Friedman, D. S., & Gazzard, G. (2023). Glaucoma: now and beyond. *Lancet*, 402(10414), 1788–1801. [https://doi.org/10.1016/s0140-6736\(23\)01289-8](https://doi.org/10.1016/s0140-6736(23)01289-8)
- Jeon, C. J., Strettoi, E., & Masland, R. H. (1998). The major cell populations of the mouse retina. *J Neurosci*, 18(21), 8936–8946. <https://doi.org/10.1523/jneurosci.18-21-08936.1998>
- Jin, N. G., Chuang, A. Z., Masson, P. J., & Ribelayga, C. P. (2015). Rod electrical coupling is controlled by a circadian clock and dopamine in mouse retina. *J Physiol*, 593(7), 1597–1631. <https://doi.org/10.1113/jphysiol.2014.284919>
- Joussen, A. M., Doehmen, S., Le, M. L., Koizumi, K., Radetzky, S., Krohne, T. U., Poulaki, V., Semkova, I., & Kociok, N. (2009). TNF-alpha mediated apoptosis plays an important role in the development of early diabetic retinopathy and long-term histopathological alterations. *Mol Vis*, 15, 1418–1428.
- Kalesnykas, G., Oglesby, E. N., Zack, D. J., Cone, F. E., Steinhart, M. R., Tian, J., Pease, M. E., & Quigley, H. A. (2012). Retinal ganglion cell morphology after optic nerve crush and experimental glaucoma. *Invest Ophthalmol Vis Sci*, 53(7), 3847–3857.
<https://doi.org/10.1167/iovs.12-9712>
- Kaneko, A. (1971). Electrical connexions between horizontal cells in the dogfish retina. *J Physiol*, 213(1), 95–105. <https://doi.org/10.1113/jphysiol.1971.sp009370>
- Kass, M. A., Heuer, D. K., Higginbotham, E. J., Johnson, C. A., Keltner, J. L., Miller, J. P., Parrish, R. K., 2nd, Wilson, M. R., & Gordon, M. O. (2002). The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive

- medication delays or prevents the onset of primary open-angle glaucoma. *Arch Ophthalmol*, 120(6), 701–713; discussion 829–730.
<https://doi.org/10.1001/archophth.120.6.701>
- Kawamura, S., & Tachibanaki, S. (2008). Rod and cone photoreceptors: molecular basis of the difference in their physiology. *Comp Biochem Physiol A Mol Integr Physiol*, 150(4), 369–377. <https://doi.org/10.1016/j.cbpa.2008.04.600>
- Keeley, P. W., & Reese, B. E. (2010). Morphology of dopaminergic amacrine cells in the mouse retina: independence from homotypic interactions. *J Comp Neurol*, 518(8), 1220–1231.
<https://doi.org/10.1002/cne.22270>
- Kim, B. J., Braun, T. A., Wordinger, R. J., & Clark, A. F. (2013). Progressive morphological changes and impaired retinal function associated with temporal regulation of gene expression after retinal ischemia/reperfusion injury in mice. *Mol Neurodegener*, 8, 21.
<https://doi.org/10.1186/1750-1326-8-21>
- Kim, U. S., Mahroo, O. A., Mollon, J. D., & Yu-Wai-Man, P. (2021). Retinal Ganglion Cells-Diversity of Cell Types and Clinical Relevance. *Front Neurol*, 12, 661938.
<https://doi.org/10.3389/fneur.2021.661938>
- Kirichenko, E. Y., Skatchkov, S. N., & Ermakov, A. M. (2021). Structure and Functions of Gap Junctions and Their Constituent Connexins in the Mammalian CNS. *Biochem (Mosc) Suppl Ser A Membr Cell Biol*, 15(2), 107–119.
<https://doi.org/10.1134/s1990747821020069>
- Klitten, L. L., Rath, M. F., Coon, S. L., Kim, J. S., Klein, D. C., & Møller, M. (2008). Localization and regulation of dopamine receptor D4 expression in the adult and

developing rat retina. *Exp Eye Res*, 87(5), 471–477.

<https://doi.org/10.1016/j.exer.2008.08.004>

Koike, C., Obara, T., Uriu, Y., Numata, T., Sanuki, R., Miyata, K., Koyasu, T., Ueno, S., Funabiki, K., Tani, A., Ueda, H., Kondo, M., Mori, Y., Tachibana, M., & Furukawa, T. (2010). TRPM1 is a component of the retinal ON bipolar cell transduction channel in the mGluR6 cascade. *Proc Natl Acad Sci U S A*, 107(1), 332–337.

<https://doi.org/10.1073/pnas.0912730107>

Koistinaho, J., Swanson, R. A., de Vente, J., & Sagar, S. M. (1993). NADPH-diaphorase (nitric oxide synthase)-reactive amacrine cells of rabbit retina: putative target cells and stimulation by light. *Neuroscience*, 57(3), 587–597. [https://doi.org/10.1016/0306-](https://doi.org/10.1016/0306-4522(93)90008-4)

[4522\(93\)90008-4](https://doi.org/10.1016/0306-4522(93)90008-4)

Kolb, H. (1995). Cone Pathways through the Retina by Helga Kolb. In H. Kolb, E. Fernandez, B. Jones, & R. Nelson (Eds.), *Webvision: The Organization of the Retina and Visual System*. University of Utah Health Sciences Center

Copyright: © 2025 Webvision .

Kolb, H., & Famiglietti, E. V. (1974). Rod and cone pathways in the inner plexiform layer of cat retina. *Science*, 186(4158), 47–49. <https://doi.org/10.1126/science.186.4158.47>

Kolb, H., Fernandez, E., Schouten, J., Ahnelt, P., Linberg, K. A., & Fisher, S. K. (1994). Are there three types of horizontal cell in the human retina? *J Comp Neurol*, 343(3), 370–386.

<https://doi.org/10.1002/cne.903430304>

Kolb, H., Nelson, R., & Mariani, A. (1981). Amacrine cells, bipolar cells and ganglion cells of the cat retina: a Golgi study. *Vision Res*, 21(7), 1081–1114. [https://doi.org/10.1016/0042-](https://doi.org/10.1016/0042-6989(81)90013-4)

[6989\(81\)90013-4](https://doi.org/10.1016/0042-6989(81)90013-4)

- Kong, J. H., Park, S. P., & Na, K. I. (2023). Differences in optic nerve head structure between acute angle-closure glaucoma and open-angle glaucoma. *Sci Rep*, 13(1), 7935.
<https://doi.org/10.1038/s41598-023-35020-y>
- Kothmann, W. W., Massey, S. C., & O'Brien, J. (2009). Dopamine-stimulated dephosphorylation of connexin 36 mediates AII amacrine cell uncoupling. *J Neurosci*, 29(47), 14903–14911.
<https://doi.org/10.1523/jneurosci.3436-09.2009>
- Kranz, K., Paquet-Durand, F., Weiler, R., Janssen-Bienhold, U., & Dedek, K. (2013). Testing for a gap junction-mediated bystander effect in retinitis pigmentosa: secondary cone death is not altered by deletion of connexin36 from cones. *PLoS One*, 8(2), e57163.
<https://doi.org/10.1371/journal.pone.0057163>
- Krieger, B., Qiao, M., Rousso, D. L., Sanes, J. R., & Meister, M. (2017). Four alpha ganglion cell types in mouse retina: Function, structure, and molecular signatures. *PLoS One*, 12(7), e0180091. <https://doi.org/10.1371/journal.pone.0180091>
- Kuffler, S. W. (1953). Discharge patterns and functional organization of mammalian retina. *J Neurophysiol*, 16(1), 37–68. <https://doi.org/10.1152/jn.1953.16.1.37>
- Kumar, S., Akopian, A., & Bloomfield, S. A. (2023). Neuroprotection of Retinal Ganglion Cells Suppresses Microglia Activation in a Mouse Model of Glaucoma. *Invest Ophthalmol Vis Sci*, 64(7), 24. <https://doi.org/10.1167/iovs.64.7.24>
- Lampe, P. D., & Lau, A. F. (2000). Regulation of gap junctions by phosphorylation of connexins. *Arch Biochem Biophys*, 384(2), 205–215. <https://doi.org/10.1006/abbi.2000.2131>
- Lee, E. J., Han, J. W., Kim, H. J., Kim, I. B., Lee, M. Y., Oh, S. J., Chung, J. W., & Chun, M. H. (2003). The immunocytochemical localization of connexin 36 at rod and cone gap

- junctions in the guinea pig retina. *Eur J Neurosci*, 18(11), 2925–2934.
<https://doi.org/10.1046/j.1460-9568.2003.03049.x>
- Lee, E. J., Mann, L. B., Rickman, D. W., Lim, E. J., Chun, M. H., & Grzywacz, N. M. (2006). All amacrine cells in the distal inner nuclear layer of the mouse retina. *J Comp Neurol*, 494(4), 651–662. <https://doi.org/10.1002/cne.20838>
- Lee, S., Zhang, Y., Chen, M., & Zhou, Z. J. (2016). Segregated Glycine-Glutamate Co-transmission from vGluT3 Amacrine Cells to Contrast-Suppressed and Contrast-Enhanced Retinal Circuits. *Neuron*, 90(1), 27–34.
<https://doi.org/10.1016/j.neuron.2016.02.023>
- Lee, S. C., Meyer, A., Schubert, T., Hüsler, L., Dedek, K., & Haverkamp, S. (2015). Morphology and connectivity of the small bistratified A8 amacrine cell in the mouse retina. *J Comp Neurol*, 523(10), 1529–1547. <https://doi.org/10.1002/cne.23752>
- Lee, S. K., & Schmidt, T. M. (2018). Morphological Identification of Melanopsin-Expressing Retinal Ganglion Cell Subtypes in Mice. *Methods Mol Biol*, 1753, 275–287.
https://doi.org/10.1007/978-1-4939-7720-8_19
- Leung, C. K., Lindsey, J. D., Chen, L., Liu, Q., & Weinreb, R. N. (2009). Longitudinal profile of retinal ganglion cell damage assessed with blue-light confocal scanning laser ophthalmoscopy after ischaemic reperfusion injury. *Br J Ophthalmol*, 93(7), 964–968.
<https://doi.org/10.1136/bjo.2008.150482>
- Li, X., Kamasawa, N., Ciolofan, C., Olson, C. O., Lu, S., Davidson, K. G., Yasumura, T., Shigemoto, R., Rash, J. E., & Nagy, J. I. (2008). Connexin45-containing neuronal gap junctions in rodent retina also contain connexin36 in both apposing hemiplaques, forming

- bihomotypic gap junctions, with scaffolding contributed by zonula occludens-1. *J Neurosci*, 28(39), 9769–9789. <https://doi.org/10.1523/jneurosci.2137-08.2008>
- Li, Z., Choi, W., Oh, H. J., & Yoon, K. C. (2012). Effectiveness of topical infliximab in a mouse model of experimental dry eye. *Cornea*, 31 Suppl 1, S25–31. <https://doi.org/10.1097/ICO.0b013e31826a80ea>
- Lin, B., & Masland, R. H. (2006). Populations of wide-field amacrine cells in the mouse retina. *J Comp Neurol*, 499(5), 797–809. <https://doi.org/10.1002/cne.21126>
- Lin, J., Ziring, D., Desai, S., Kim, S., Wong, M., Korin, Y., Braun, J., Reed, E., Gjertson, D., & Singh, R. R. (2008). TNFalpha blockade in human diseases: an overview of efficacy and safety. *Clin Immunol*, 126(1), 13–30. <https://doi.org/10.1016/j.clim.2007.08.012>
- Lin, M. P., Ekşioğlu, Ü., Mudumbai, R. C., Slabaugh, M. A., & Chen, P. P. (2012). Glaucoma in patients with ocular chemical burns. *Am J Ophthalmol*, 154(3), 481–485.e481. <https://doi.org/10.1016/j.ajo.2012.03.026>
- Liu, Z., Saeedi, O., Zhang, F., Villanueva, R., Asanad, S., Agrawal, A., & Hammer, D. X. (2021). Quantification of Retinal Ganglion Cell Morphology in Human Glaucomatous Eyes. *Invest Ophthalmol Vis Sci*, 62(3), 34. <https://doi.org/10.1167/iovs.62.3.34>
- Lopez-Gonzalez, R., Loza, E., Jover, J. A., Benitez Del Castillo, J. M., Mendez, R., Hernandez-Garcia, C., & Pato, E. (2009). Treatment of refractory posterior uveitis with infliximab: a 7-year follow-up study. *Scand J Rheumatol*, 38(1), 58–62. <https://doi.org/10.1080/03009740802366076>
- Lyubarsky, A. L., Falsini, B., Pennesi, M. E., Valentini, P., & Pugh, E. N., Jr. (1999). UV- and midwave-sensitive cone-driven retinal responses of the mouse: a possible phenotype for

- coexpression of cone photopigments. *J Neurosci*, 19(1), 442–455.
<https://doi.org/10.1523/jneurosci.19-01-00442.1999>
- Maini, R. N., & Feldmann, M. (2002). How does infliximab work in rheumatoid arthritis? *Arthritis Research & Therapy*, 4(2), S22. <https://doi.org/10.1186/ar549>
- Manookin, M. B., Beaudoin, D. L., Ernst, Z. R., Flagel, L. J., & Demb, J. B. (2008).
 Disinhibition combines with excitation to extend the operating range of the OFF visual pathway in daylight. *J Neurosci*, 28(16), 4136–4150.
<https://doi.org/10.1523/jneurosci.4274-07.2008>
- Marc, R. E., & Liu, W. (2000). Fundamental GABAergic amacrine cell circuitries in the retina: nested feedback, concatenated inhibition, and axosomatic synapses. *J Comp Neurol*, 425(4), 560–582. [https://doi.org/10.1002/1096-9861\(20001002\)425:4<560::aid-cne7>3.0.co;2-d](https://doi.org/10.1002/1096-9861(20001002)425:4<560::aid-cne7>3.0.co;2-d)
- Masland, R. H. (2001). The fundamental plan of the retina. *Nat Neurosci*, 4(9), 877–886.
<https://doi.org/10.1038/nn0901-877>
- Masland, R. H. (2012a). The neuronal organization of the retina. *Neuron*, 76(2), 266–280.
<https://doi.org/10.1016/j.neuron.2012.10.002>
- Masland, R. H. (2012b). The tasks of amacrine cells. *Vis Neurosci*, 29(1), 3–9.
<https://doi.org/10.1017/s0952523811000344>
- Massey, S. C., O'Brien, J. J., Trexler, E. B., Li, W., Keung, J. W., Mills, S. L., & O'Brien, J. (2003). Multiple neuronal connexins in the mammalian retina. *Cell Commun Adhes*, 10(4-6), 425–430. <https://doi.org/10.1080/cac.10.4-6.425.430>

- Mataruga, A., Kremmer, E., & Müller, F. (2007). Type 3a and type 3b OFF cone bipolar cells provide for the alternative rod pathway in the mouse retina. *J Comp Neurol*, 502(6), 1123–1137. <https://doi.org/10.1002/cne.21367>
- McDermott, M. F. (2001). TNF and TNFR biology in health and disease. *Cell Mol Biol (Noisy-le-grand)*, 47(4), 619–635.
- Menger, N., Pow, D. V., & Wässle, H. (1998). Glycinergic amacrine cells of the rat retina. *J Comp Neurol*, 401(1), 34–46. [https://doi.org/10.1002/\(sici\)1096-9861\(19981109\)401:1<34::aid-cne3>3.0.co;2-p](https://doi.org/10.1002/(sici)1096-9861(19981109)401:1<34::aid-cne3>3.0.co;2-p)
- Mills, S. L., O'Brien, J. J., Li, W., O'Brien, J., & Massey, S. C. (2001). Rod pathways in the mammalian retina use connexin 36. *J Comp Neurol*, 436(3), 336–350.
- Miyamoto, F., Sotozono, C., Ikeda, T., & Kinoshita, S. (1998). Retinal cytokine response in mouse alkali-burned eye. *Ophthalmic Res*, 30(3), 168–171. <https://doi.org/10.1159/000055470>
- Moreno, A. P., de Carvalho, A. C., Verselis, V., Eghbali, B., & Spray, D. C. (1991). Voltage-dependent gap junction channels are formed by connexin32, the major gap junction protein of rat liver. *Biophys J*, 59(4), 920–925. [https://doi.org/10.1016/s0006-3495\(91\)82305-0](https://doi.org/10.1016/s0006-3495(91)82305-0)
- Morozumi, W., Inagaki, S., Iwata, Y., Nakamura, S., Hara, H., & Shimazawa, M. (2020). Piezo channel plays a part in retinal ganglion cell damage. *Exp Eye Res*, 191, 107900. <https://doi.org/10.1016/j.exer.2019.107900>
- Müller, L. P., Dedek, K., Janssen-Bienhold, U., Meyer, A., Kreuzberg, M. M., Lorenz, S., Willecke, K., & Weiler, R. (2010). Expression and modulation of connexin 30.2, a novel

- gap junction protein in the mouse retina. *Vis Neurosci*, 27(3-4), 91–101.
<https://doi.org/10.1017/s0952523810000131>
- Münch, T. A., da Silveira, R. A., Siebert, S., Viney, T. J., Awatramani, G. B., & Roska, B. (2009). Approach sensitivity in the retina processed by a multifunctional neural circuit. *Nat Neurosci*, 12(10), 1308–1316. <https://doi.org/10.1038/nn.2389>
- Murphy, C. C., Ayliffe, W. H., Booth, A., Mäkelä, D., Andrews, P. A., & Jayne, D. (2004). Tumor necrosis factor alpha blockade with infliximab for refractory uveitis and scleritis. *Ophthalmology*, 111(2), 352–356. [https://doi.org/10.1016/s0161-6420\(03\)00721-8](https://doi.org/10.1016/s0161-6420(03)00721-8)
- Murphy, G. J., & Rieke, F. (2008). Signals and noise in an inhibitory interneuron diverge to control activity in nearby retinal ganglion cells. *Nat Neurosci*, 11(3), 318–326.
<https://doi.org/10.1038/nn2045>
- Nelson, R., Famiglietti, E. V., Jr., & Kolb, H. (1978). Intracellular staining reveals different levels of stratification for on- and off-center ganglion cells in cat retina. *J Neurophysiol*, 41(2), 472–483. <https://doi.org/10.1152/jn.1978.41.2.472>
- Nicholson, B. J., Weber, P. A., Cao, F., Chang, H., Lampe, P., & Goldberg, G. (2000). The molecular basis of selective permeability of connexins is complex and includes both size and charge. *Braz J Med Biol Res*, 33(4), 369–378. <https://doi.org/10.1590/s0100-879x2000000400002>
- O'Brien, J. (2014). The ever-changing electrical synapse. *Curr Opin Neurobiol*, 29, 64–72.
<https://doi.org/10.1016/j.conb.2014.05.011>
- O'Brien, J., & Bloomfield, S. A. (2018). Plasticity of Retinal Gap Junctions: Roles in Synaptic Physiology and Disease. *Annu Rev Vis Sci*, 4, 79–100. <https://doi.org/10.1146/annurev-vision-091517-034133>

- O'Brien, J. J., Li, W., Pan, F., Keung, J., O'Brien, J., & Massey, S. C. (2006). Coupling between A-type horizontal cells is mediated by connexin 50 gap junctions in the rabbit retina. *J Neurosci*, 26(45), 11624–11636. <https://doi.org/10.1523/jneurosci.2296-06.2006>
- Oltedal, L., Mørkve, S. H., Veruki, M. L., & Hartveit, E. (2007). Patch-clamp investigations and compartmental modeling of rod bipolar axon terminals in an in vitro thin-slice preparation of the mammalian retina. *J Neurophysiol*, 97(2), 1171–1187. <https://doi.org/10.1152/jn.01010.2006>
- Ou, Y., Jo, R. E., Ullian, E. M., Wong, R. O., & Della Santina, L. (2016). Selective Vulnerability of Specific Retinal Ganglion Cell Types and Synapses after Transient Ocular Hypertension. *J Neurosci*, 36(35), 9240–9252. <https://doi.org/10.1523/jneurosci.0940-16.2016>
- Pan, F., Keung, J., Kim, I. B., Snuggs, M. B., Mills, S. L., O'Brien, J., & Massey, S. C. (2012). Connexin 57 is expressed by the axon terminal network of B-type horizontal cells in the rabbit retina. *J Comp Neurol*, 520(10), 2256–2274. <https://doi.org/10.1002/cne.23060>
- Pan, F., Paul, D. L., Bloomfield, S. A., & Völgyi, B. (2010). Connexin36 is required for gap junctional coupling of most ganglion cell subtypes in the mouse retina. *J Comp Neurol*, 518(6), 911–927. <https://doi.org/10.1002/cne.22254>
- Pan, F., Toychiev, A., Zhang, Y., Atlasz, T., Ramakrishnan, H., Roy, K., Völgyi, B., Akopian, A., & Bloomfield, S. A. (2016). Inhibitory masking controls the threshold sensitivity of retinal ganglion cells. *J Physiol*, 594(22), 6679–6699. <https://doi.org/10.1113/jp272267>
- Pang, I. H., & Clark, A. F. (2020). Inducible rodent models of glaucoma. *Prog Retin Eye Res*, 75, 100799. <https://doi.org/10.1016/j.preteyeres.2019.100799>

- Pang, J. J. (2024). The Variety of Mechanosensitive Ion Channels in Retinal Neurons. *Int J Mol Sci*, 25(9). <https://doi.org/10.3390/ijms25094877>
- Pang, J. J., Frankfort, B. J., Gross, R. L., & Wu, S. M. (2015). Elevated intraocular pressure decreases response sensitivity of inner retinal neurons in experimental glaucoma mice. *Proc Natl Acad Sci U S A*, 112(8), 2593–2598. <https://doi.org/10.1073/pnas.1419921112>
- Pang, J. J., Gao, F., & Wu, S. M. (2003). Light-evoked excitatory and inhibitory synaptic inputs to ON and OFF alpha ganglion cells in the mouse retina. *J Neurosci*, 23(14), 6063–6073. <https://doi.org/10.1523/jneurosci.23-14-06063.2003>
- Pang, J. J., Gao, F., & Wu, S. M. (2012). Physiological characterization and functional heterogeneity of narrow-field mammalian amacrine cells. *J Physiol*, 590(1), 223–234. <https://doi.org/10.1113/jphysiol.2011.222141>
- Pang, J. J., Paul, D. L., & Wu, S. M. (2013). Survey on amacrine cells coupling to retrograde-identified ganglion cells in the mouse retina. *Invest Ophthalmol Vis Sci*, 54(8), 5151–5162. <https://doi.org/10.1167/iovs.13-11774>
- Park, H. Y., Kim, J. H., & Park, C. K. (2014). Alterations of the synapse of the inner retinal layers after chronic intraocular pressure elevation in glaucoma animal model. *Mol Brain*, 7, 53. <https://doi.org/10.1186/s13041-014-0053-2>
- Paschalis, E. I., Lei, F., Zhou, C., Kapoulea, V., Thanos, A., Dana, R., Vavvas, D. G., Chodosh, J., & Dohlman, C. H. (2018). The Role of Microglia and Peripheral Monocytes in Retinal Damage after Corneal Chemical Injury. *Am J Pathol*, 188(7), 1580–1596. <https://doi.org/10.1016/j.ajpath.2018.03.005>
- Paschalis, E. I., Zhou, C., Lei, F., Scott, N., Kapoulea, V., Robert, M. C., Vavvas, D., Dana, R., Chodosh, J., & Dohlman, C. H. (2017). Mechanisms of Retinal Damage after Ocular

- Alkali Burns. *Am J Pathol*, 187(6), 1327–1342.
<https://doi.org/10.1016/j.ajpath.2017.02.005>
- Peek, M. Y., & Card, G. M. (2016). Comparative approaches to escape. *Curr Opin Neurobiol*, 41, 167–173. <https://doi.org/10.1016/j.conb.2016.09.012>
- Peichl, L., & González-Soriano, J. (1994). Morphological types of horizontal cell in rodent retinae: a comparison of rat, mouse, gerbil, and guinea pig. *Vis Neurosci*, 11(3), 501–517.
<https://doi.org/10.1017/s095252380000242x>
- Peichl, L., Sandmann, D., & Boycott, B. B. (1998). Comparative Anatomy and Function of Mammalian Horizontal Cells. In L. M. Chalupa & B. L. Finlay (Eds.), *Development and Organization of the Retina: From Molecules to function* (pp. 147–172). Springer US.
https://doi.org/10.1007/978-1-4615-5333-5_9
- Peichl, L., & Wässle, H. (1981). Morphological identification of on- and off-centre brisk transient (Y) cells in the cat retina. *Proc R Soc Lond B Biol Sci*, 212(1187), 139–153.
<https://doi.org/10.1098/rspb.1981.0030>
- Peirson, S. N., Brown, L. A., Pothecary, C. A., Benson, L. A., & Fisk, A. S. (2018). Light and the laboratory mouse. *J Neurosci Methods*, 300, 26–36.
<https://doi.org/10.1016/j.jneumeth.2017.04.007>
- Pérez de Sevilla Müller, L., Azar, S. S., de Los Santos, J., & Brecha, N. C. (2017). Prox1 Is a Marker for All Amacrine Cells in the Mouse Retina. *Front Neuroanat*, 11, 39.
<https://doi.org/10.3389/fnana.2017.00039>
- Pickard, G. E., & Sollars, P. J. (2012). Intrinsically photosensitive retinal ganglion cells. *Rev Physiol Biochem Pharmacol*, 162, 59–90. https://doi.org/10.1007/112_2011_4

- Planck, S. R., Rich, L. F., Ansel, J. C., Huang, X. N., & Rosenbaum, J. T. (1997). Trauma and alkali burns induce distinct patterns of cytokine gene expression in the rat cornea. *Ocul Immunol Inflamm*, 5(2), 95–100. <https://doi.org/10.3109/09273949709085057>
- Poleg-Polsky, A., & Diamond, J. S. (2011). Imperfect space clamp permits electrotonic interactions between inhibitory and excitatory synaptic conductances, distorting voltage clamp recordings. *PLoS One*, 6(4), e19463. <https://doi.org/10.1371/journal.pone.0019463>
- Popova, E. (1995). Role of Dopamine in Retinal Function. In H. Kolb, E. Fernandez, B. Jones, & R. Nelson (Eds.), *Webvision: The Organization of the Retina and Visual System*. University of Utah Health Sciences Center
- Copyright: © 2025 Webvision .
- Pourcho, R. G., & Goebel, D. J. (1985). Immunocytochemical demonstration of glycine in retina. *Brain Res*, 348(2), 339–342. [https://doi.org/10.1016/0006-8993\(85\)90453-6](https://doi.org/10.1016/0006-8993(85)90453-6)
- Qiu, X., Kumbalasiri, T., Carlson, S. M., Wong, K. Y., Krishna, V., Provencio, I., & Berson, D. M. (2005). Induction of photosensitivity by heterologous expression of melanopsin. *Nature*, 433(7027), 745–749. <https://doi.org/10.1038/nature03345>
- Quesada, J. M., Lloves, J. M., & Delgado, D. V. (2020). Ocular chemical burns in the workplace: Epidemiological characteristics. *Burns*, 46(5), 1212–1218. <https://doi.org/10.1016/j.burns.2019.11.007>
- Quigley, H. A., & Addicks, E. M. (1980). Chronic experimental glaucoma in primates. II. Effect of extended intraocular pressure elevation on optic nerve head and axonal transport. *Invest Ophthalmol Vis Sci*, 19(2), 137–152.

- Quigley, H. A., & Broman, A. T. (2006). The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*, 90(3), 262–267.
<https://doi.org/10.1136/bjo.2005.081224>
- Ran, Y., Huang, Z., Baden, T., Schubert, T., Baayen, H., Berens, P., Franke, K., & Euler, T. (2020). Type-specific dendritic integration in mouse retinal ganglion cells. *Nat Commun*, 11(1), 2101. <https://doi.org/10.1038/s41467-020-15867-9>
- Ransohoff, R. M., & Brown, M. A. (2012). Innate immunity in the central nervous system. *J Clin Invest*, 122(4), 1164–1171. <https://doi.org/10.1172/jci58644>
- Rheume, B. A., Jereen, A., Bolisetty, M., Sajid, M. S., Yang, Y., Renna, K., Sun, L., Robson, P., & Trakhtenberg, E. F. (2018). Single cell transcriptome profiling of retinal ganglion cells identifies cellular subtypes. *Nat Commun*, 9(1), 2759. <https://doi.org/10.1038/s41467-018-05134-3>
- Ribelayga, C., Cao, Y., & Mangel, S. C. (2008). The circadian clock in the retina controls rod-cone coupling. *Neuron*, 59(5), 790–801. <https://doi.org/10.1016/j.neuron.2008.07.017>
- Rice, D. S., & Curran, T. (2000). Disabled-1 is expressed in type AII amacrine cells in the mouse retina. *J Comp Neurol*, 424(2), 327–338. [https://doi.org/10.1002/1096-9861\(20000821\)424:2<327::aid-cne10>3.0.co;2-6](https://doi.org/10.1002/1096-9861(20000821)424:2<327::aid-cne10>3.0.co;2-6)
- Ripps, H. (2002). Cell death in retinitis pigmentosa: gap junctions and the 'bystander' effect. *Exp Eye Res*, 74(3), 327–336. <https://doi.org/10.1006/exer.2002.1155>
- Risner, M. L., Pasini, S., Cooper, M. L., Lambert, W. S., & Calkins, D. J. (2018). Axogenic mechanism enhances retinal ganglion cell excitability during early progression in glaucoma. *Proc Natl Acad Sci U S A*, 115(10), E2393–e2402.
<https://doi.org/10.1073/pnas.1714888115>

- Robert, M. C., Črnej, A., Shen, L. Q., Papaliodis, G. N., Dana, R., Foster, C. S., Chodosh, J., & Dohlman, C. H. (2017). Infliximab after Boston Keratoprosthesis in Stevens-Johnson Syndrome: An Update. *Ocul Immunol Inflamm*, 25(3), 413–417.
<https://doi.org/10.3109/09273948.2016.1145237>
- Rodrigues, E. B., Farah, M. E., Maia, M., Penha, F. M., Regatieri, C., Melo, G. B., Pinheiro, M. M., & Zanetti, C. R. (2009). Therapeutic monoclonal antibodies in ophthalmology. *Prog Retin Eye Res*, 28(2), 117–144. <https://doi.org/10.1016/j.preteyeres.2008.11.005>
- Roh, M., Zhang, Y., Murakami, Y., Thanos, A., Lee, S. C., Vavvas, D. G., Benowitz, L. I., & Miller, J. W. (2012). Etanercept, a widely used inhibitor of tumor necrosis factor- α (TNF- α), prevents retinal ganglion cell loss in a rat model of glaucoma. *PLoS One*, 7(7), e40065. <https://doi.org/10.1371/journal.pone.0040065>
- Roper-Hall, M. J. (1965). Thermal and chemical burns. *Trans Ophthalmol Soc U K (1962)*, 85, 631–653.
- Roska, B., & Werblin, F. (2001). Vertical interactions across ten parallel, stacked representations in the mammalian retina. *Nature*, 410(6828), 583–587. <https://doi.org/10.1038/35069068>
- Sakai, H. M., & Naka, K. I. (1990). Dissection of the neuron network in the catfish inner retina. IV. Bidirectional interactions between amacrine and ganglion cells. *J Neurophysiol*, 63(1), 105–119. <https://doi.org/10.1152/jn.1990.63.1.105>
- Salinas-Navarro, M., Alarcón-Martínez, L., Valiente-Soriano, F. J., Ortín-Martínez, A., Jiménez-López, M., Avilés-Trigueros, M., Villegas-Pérez, M. P., de la Villa, P., & Vidal-Sanz, M. (2009). Functional and morphological effects of laser-induced ocular hypertension in retinas of adult albino Swiss mice. *Mol Vis*, 15, 2578–2598.

- Sanes, J. R., & Masland, R. H. (2015). The types of retinal ganglion cells: current status and implications for neuronal classification. *Annu Rev Neurosci*, 38, 221–246.
<https://doi.org/10.1146/annurev-neuro-071714-034120>
- Schachter, M. J., Oesch, N., Smith, R. G., & Taylor, W. R. (2010). Dendritic spikes amplify the synaptic signal to enhance detection of motion in a simulation of the direction-selective ganglion cell. *PLoS Comput Biol*, 6(8). <https://doi.org/10.1371/journal.pcbi.1000899>
- Schaller, B., Graf, R., & Jacobs, A. H. (2003). Ischaemic tolerance: a window to endogenous neuroprotection? *Lancet*, 362(9389), 1007–1008. [https://doi.org/10.1016/s0140-6736\(03\)14446-7](https://doi.org/10.1016/s0140-6736(03)14446-7)
- Schaller, B. J., Bahr, M., & Buchfelder, M. (2005). Pathophysiology of brain ischemia: penumbra, gene expression, and future therapeutic options. *Eur Neurol*, 54(4), 179–180.
<https://doi.org/10.1159/000090293>
- Schneider, M., & Fuchshofer, R. (2016). The role of astrocytes in optic nerve head fibrosis in glaucoma. *Exp Eye Res*, 142, 49–55. <https://doi.org/10.1016/j.exer.2015.08.014>
- Schrage, N. F., Langefeld, S., Zschocke, J., Kuckelkorn, R., Redbrake, C., & Reim, M. (2000). Eye burns: an emergency and continuing problem. *Burns*, 26(8), 689–699.
[https://doi.org/10.1016/s0305-4179\(00\)00044-9](https://doi.org/10.1016/s0305-4179(00)00044-9)
- Schubert, T., Degen, J., Willecke, K., Hormuzdi, S. G., Monyer, H., & Weiler, R. (2005). Connexin36 mediates gap junctional coupling of alpha-ganglion cells in mouse retina. *J Comp Neurol*, 485(3), 191–201. <https://doi.org/10.1002/cne.20510>
- Schubert, T., Maxeiner, S., Krüger, O., Willecke, K., & Weiler, R. (2005). Connexin45 mediates gap junctional coupling of bistratified ganglion cells in the mouse retina. *J Comp Neurol*, 490(1), 29–39. <https://doi.org/10.1002/cne.20621>

- Schwartz, M., & Yoles, E. (1999). Optic nerve degeneration and potential neuroprotection: implications for glaucoma. *Eur J Ophthalmol*, 9 Suppl 1, S9–11.
<https://doi.org/10.1177/112067219900901s07>
- Sellés-Navarro, I., Villegas-Pérez, M. P., Salvador-Silva, M., Ruiz-Gómez, J. M., & Vidal-Sanz, M. (1996). Retinal ganglion cell death after different transient periods of pressure-induced ischemia and survival intervals. A quantitative in vivo study. *Invest Ophthalmol Vis Sci*, 37(10), 2002–2014.
- Sharp, P. S. (1995). The role of growth factors in the development of diabetic retinopathy. *Metabolism*, 44(10 Suppl 4), 72–75. [https://doi.org/10.1016/0026-0495\(95\)90224-4](https://doi.org/10.1016/0026-0495(95)90224-4)
- Shelley, J., Dedek, K., Schubert, T., Feigenspan, A., Schultz, K., Hombach, S., Willecke, K., & Weiler, R. (2006). Horizontal cell receptive fields are reduced in connexin57-deficient mice. *Eur J Neurosci*, 23(12), 3176–3186. <https://doi.org/10.1111/j.1460-9568.2006.04848.x>
- Silverman, S. M., & Wong, W. T. (2018). Microglia in the Retina: Roles in Development, Maturity, and Disease. *Annu Rev Vis Sci*, 4, 45–77. <https://doi.org/10.1146/annurev-vision-091517-034425>
- Simpson, I., Rose, B., & Loewenstein, W. R. (1977). Size limit of molecules permeating the junctional membrane channels. *Science*, 195(4275), 294–296.
<https://doi.org/10.1126/science.831276>
- Singh, P., Tyagi, M., Kumar, Y., Gupta, K. K., & Sharma, P. D. (2013). Ocular chemical injuries and their management. *Oman J Ophthalmol*, 6(2), 83–86. <https://doi.org/10.4103/0974-620x.116624>

- Söhl, G., Joussen, A., Kociok, N., & Willecke, K. (2010). Expression of connexin genes in the human retina. *BMC Ophthalmol*, 10, 27. <https://doi.org/10.1186/1471-2415-10-27>
- Söhl, G., Maxeiner, S., & Willecke, K. (2005). Expression and functions of neuronal gap junctions. *Nat Rev Neurosci*, 6(3), 191–200. <https://doi.org/10.1038/nrn1627>
- Söhl, G., Odermatt, B., Maxeiner, S., Degen, J., & Willecke, K. (2004). New insights into the expression and function of neural connexins with transgenic mouse mutants. *Brain Res Brain Res Rev*, 47(1-3), 245–259. <https://doi.org/10.1016/j.brainresrev.2004.05.006>
- Soleimani, M., & Naderan, M. (2020). Management Strategies of Ocular Chemical Burns: Current Perspectives. *Clin Ophthalmol*, 14, 2687–2699. <https://doi.org/10.2147/opth.S235873>
- Sotozono, C., He, J., Matsumoto, Y., Kita, M., Imanishi, J., & Kinoshita, S. (1997). Cytokine expression in the alkali-burned cornea. *Curr Eye Res*, 16(7), 670–676. <https://doi.org/10.1076/ceyr.16.7.670.5057>
- Srinivas, M., Costa, M., Gao, Y., Fort, A., Fishman, G. I., & Spray, D. C. (1999). Voltage dependence of macroscopic and unitary currents of gap junction channels formed by mouse connexin50 expressed in rat neuroblastoma cells. *J Physiol*, 517 (Pt 3)(Pt 3), 673–689. <https://doi.org/10.1111/j.1469-7793.1999.0673s.x>
- Sripinun, P., See, L. P., Nikonov, S., Chavali, V. R. M., Vratasha, V., He, J., O'Brien, J. M., Xia, J., Lu, W., & Mitchell, C. H. (2024). Piezo1 and Piezo2 channels in retinal ganglion cells and the impact of Piezo1 stimulation on light-dependent neural activity. *bioRxiv*. <https://doi.org/10.1101/2024.06.25.599602>

- Streit, M., Beleznay, Z., & Braathen, L. R. (2006). Topical application of the tumour necrosis factor-alpha antibody infliximab improves healing of chronic wounds. *Int Wound J*, 3(3), 171–179. <https://doi.org/10.1111/j.1742-481X.2006.00233.x>
- Sun, H., Macke, J. P., & Nathans, J. (1997). Mechanisms of spectral tuning in the mouse green cone pigment. *Proc Natl Acad Sci U S A*, 94(16), 8860–8865. <https://doi.org/10.1073/pnas.94.16.8860>
- Tachibana, M., & Kaneko, A. (1988). Retinal bipolar cells receive negative feedback input from GABAergic amacrine cells. *Vis Neurosci*, 1(3), 297–305. <https://doi.org/10.1017/s0952523800001954>
- Takao, M., Wang, Y., Miyoshi, T., Fujita, I., & Fukuda, Y. (2000). A new intraretinal recording system with multiple-barreled electrodes for pharmacological studies on cat retinal ganglion cells. *J Neurosci Methods*, 97(1), 87–92. [https://doi.org/10.1016/s0165-0270\(00\)00171-0](https://doi.org/10.1016/s0165-0270(00)00171-0)
- Tan, H., Li, X., Huang, K., Luo, M., & Wang, L. (2022). Morphological and distributional properties of SMI-32 immunoreactive ganglion cells in the rat retina. *J Comp Neurol*, 530(8), 1276–1287. <https://doi.org/10.1002/cne.25275>
- Tham, Y. C., Li, X., Wong, T. Y., Quigley, H. A., Aung, T., & Cheng, C. Y. (2014). Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology*, 121(11), 2081–2090. <https://doi.org/10.1016/j.ophtha.2014.05.013>
- Theis, M., Jauch, R., Zhuo, L., Speidel, D., Wallraff, A., Döring, B., Frisch, C., Söhl, G., Teubner, B., Euwens, C., Huston, J., Steinhäuser, C., Messing, A., Heinemann, U., & Willecke, K. (2003). Accelerated hippocampal spreading depression and enhanced

- locomotory activity in mice with astrocyte-directed inactivation of connexin43. *J Neurosci*, 23(3), 766–776. <https://doi.org/10.1523/jneurosci.23-03-00766.2003>
- Theodossiadis, P. G., Markomichelakis, N. N., & Sfikakis, P. P. (2007). Tumor necrosis factor antagonists: preliminary evidence for an emerging approach in the treatment of ocular inflammation. *Retina*, 27(4), 399–413. <https://doi.org/10.1097/MAJ.0b013e3180318fbc>
- Thoreson, W. B., & Mangel, S. C. (2012). Lateral interactions in the outer retina. *Prog Retin Eye Res*, 31(5), 407–441. <https://doi.org/10.1016/j.preteyeres.2012.04.003>
- Tran, N. M., Shekhar, K., Whitney, I. E., Jacobi, A., Benhar, I., Hong, G., Yan, W., Adiconis, X., Arnold, M. E., Lee, J. M., Levin, J. Z., Lin, D., Wang, C., Lieber, C. M., Regev, A., He, Z., & Sanes, J. R. (2019). Single-Cell Profiles of Retinal Ganglion Cells Differing in Resilience to Injury Reveal Neuroprotective Genes. *Neuron*, 104(6), 1039–1055.e1012. <https://doi.org/10.1016/j.neuron.2019.11.006>
- Vaney, D. I., Sivy, B., & Taylor, W. R. (2012). Direction selectivity in the retina: symmetry and asymmetry in structure and function. *Nat Rev Neurosci*, 13(3), 194–208. <https://doi.org/10.1038/nrn3165>
- Vermeire, S., Noman, M., Van Assche, G., Baert, F., Van Steen, K., Esters, N., Joossens, S., Bossuyt, X., & Rutgeerts, P. (2003). Autoimmunity associated with anti-tumor necrosis factor alpha treatment in Crohn's disease: a prospective cohort study. *Gastroenterology*, 125(1), 32–39. [https://doi.org/10.1016/s0016-5085\(03\)00701-7](https://doi.org/10.1016/s0016-5085(03)00701-7)
- Veruki, M. L., & Wässle, H. (1996). Immunohistochemical localization of dopamine D1 receptors in rat retina. *Eur J Neurosci*, 8(11), 2286–2297. <https://doi.org/10.1111/j.1460-9568.1996.tb01192.x>

- Völgyi, B., Abrams, J., Paul, D. L., & Bloomfield, S. A. (2005). Morphology and tracer coupling pattern of alpha ganglion cells in the mouse retina. *J Comp Neurol*, 492(1), 66–77.
<https://doi.org/10.1002/cne.20700>
- Völgyi, B., Chheda, S., & Bloomfield, S. A. (2009). Tracer coupling patterns of the ganglion cell subtypes in the mouse retina. *J Comp Neurol*, 512(5), 664–687.
<https://doi.org/10.1002/cne.21912>
- Völgyi, B., Deans, M. R., Paul, D. L., & Bloomfield, S. A. (2004). Convergence and segregation of the multiple rod pathways in mammalian retina. *J Neurosci*, 24(49), 11182–11192.
<https://doi.org/10.1523/jneurosci.3096-04.2004>
- Völgyi, B., Pan, F., Paul, D. L., Wang, J. T., Huberman, A. D., & Bloomfield, S. A. (2013). Gap junctions are essential for generating the correlated spike activity of neighboring retinal ganglion cells. *PLoS One*, 8(7), e69426. <https://doi.org/10.1371/journal.pone.0069426>
- Wagoner, M. D. (1997). Chemical injuries of the eye: current concepts in pathophysiology and therapy. *Surv Ophthalmol*, 41(4), 275–313. [https://doi.org/10.1016/s0039-6257\(96\)00007-0](https://doi.org/10.1016/s0039-6257(96)00007-0)
- Wallace, D. M., & O'Brien, C. J. (2016). The role of lamina cribrosa cells in optic nerve head fibrosis in glaucoma. *Exp Eye Res*, 142, 102–109.
<https://doi.org/10.1016/j.exer.2014.12.006>
- Wang, F., Li, E., De, L., Wu, Q., & Zhang, Y. (2021). OFF-transient alpha RGCs mediate looming triggered innate defensive response. *Curr Biol*, 31(11), 2263–2273.e2263.
<https://doi.org/10.1016/j.cub.2021.03.025>

- Wang, H. H., Cuenca, N., & Kolb, H. (1990). Development of morphological types and distribution patterns of amacrine cells immunoreactive to tyrosine hydroxylase in the cat retina. *Vis Neurosci*, 4(2), 159–175. <https://doi.org/10.1017/s0952523800002315>
- Ward, N. J., Ho, K. W., Lambert, W. S., Weitlauf, C., & Calkins, D. J. (2014). Absence of transient receptor potential vanilloid-1 accelerates stress-induced axonopathy in the optic projection. *J Neurosci*, 34(9), 3161–3170. <https://doi.org/10.1523/jneurosci.4089-13.2014>
- Wässle, H. (2004). Parallel processing in the mammalian retina. *Nat Rev Neurosci*, 5(10), 747–757. <https://doi.org/10.1038/nrn1497>
- Wässle, H., Puller, C., Müller, F., & Haverkamp, S. (2009). Cone contacts, mosaics, and territories of bipolar cells in the mouse retina. *J Neurosci*, 29(1), 106–117. <https://doi.org/10.1523/jneurosci.4442-08.2009>
- Westekemper, H., Figueiredo, F. C., Siah, W. F., Wagner, N., Steuhl, K. P., & Meller, D. (2017). Clinical outcomes of amniotic membrane transplantation in the management of acute ocular chemical injury. *Br J Ophthalmol*, 101(2), 103–107. <https://doi.org/10.1136/bjophthalmol-2015-308037>
- Wiggs, J. L., & Pasquale, L. R. (2017). Genetics of glaucoma. *Hum Mol Genet*, 26(R1), R21–r27. <https://doi.org/10.1093/hmg/ddx184>
- Willecke, K., Eiberger, J., Degen, J., Eckardt, D., Romualdi, A., Güldenagel, M., Deutsch, U., & Söhl, G. (2002). Structural and functional diversity of connexin genes in the mouse and human genome. *Biol Chem*, 383(5), 725–737. <https://doi.org/10.1515/bc.2002.076>
- Wong, M., Ziring, D., Korin, Y., Desai, S., Kim, S., Lin, J., Gjertson, D., Braun, J., Reed, E., & Singh, R. R. (2008). TNFalpha blockade in human diseases: mechanisms and future directions. *Clin Immunol*, 126(2), 121–136. <https://doi.org/10.1016/j.clim.2007.08.013>

- Wu, S. M. (2010). Synaptic organization of the vertebrate retina: general principles and species-specific variations: the Friedenwald lecture. *Invest Ophthalmol Vis Sci*, 51(3), 1263–1274. <https://doi.org/10.1167/iovs.09-4396>
- Xin, D., & Bloomfield, S. A. (1997). Tracer coupling pattern of amacrine and ganglion cells in the rabbit retina. *J Comp Neurol*, 383(4), 512–528. [https://doi.org/10.1002/\(sici\)1096-9861\(19970714\)383:4<512::aid-cne8>3.0.co;2-5](https://doi.org/10.1002/(sici)1096-9861(19970714)383:4<512::aid-cne8>3.0.co;2-5)
- Xin, D., & Bloomfield, S. A. (1999). Dark- and light-induced changes in coupling between horizontal cells in mammalian retina. *J Comp Neurol*, 405(1), 75–87. [https://doi.org/10.1002/\(sici\)1096-9861\(19990301\)405:1<75::aid-cne6>3.0.co;2-d](https://doi.org/10.1002/(sici)1096-9861(19990301)405:1<75::aid-cne6>3.0.co;2-d)
- Xin, D., & Bloomfield, S. A. (2000). Effects of nitric oxide on horizontal cells in the rabbit retina. *Vis Neurosci*, 17(5), 799–811. <https://doi.org/10.1017/s0952523800175133>
- Xuan, M., Wang, W., Bulloch, G., Zhang, J., Ha, J., Wang, Q., Wang, J., Lin, X., & He, M. (2024). Impact of Acute Ocular Hypertension on Retinal Ganglion Cell Loss in Mice. *Transl Vis Sci Technol*, 13(3), 17. <https://doi.org/10.1167/tvst.13.3.17>
- Yan, X., Tezel, G., Wax, M. B., & Edward, D. P. (2000). Matrix metalloproteinases and tumor necrosis factor alpha in glaucomatous optic nerve head. *Arch Ophthalmol*, 118(5), 666–673. <https://doi.org/10.1001/archopht.118.5.666>
- Yang, G. Y., Liu, F. Y., Li, X., Zhu, Q. R., Chen, B. J., & Liu, L. Q. (2019). Decreased expression of gap junction delta-2 (GJD2) messenger RNA and connexin 36 protein in form-deprivation myopia of guinea pigs. *Chin Med J (Engl)*, 132(14), 1700–1705. <https://doi.org/10.1097/cm9.0000000000000319>
- Yang, S., Zhou, J., & Li, D. (2021). Functions and Diseases of the Retinal Pigment Epithelium. *Front Pharmacol*, 12, 727870. <https://doi.org/10.3389/fphar.2021.727870>

- Zhang, A. J., Zhang, J., & Wu, S. M. (2006). Electrical coupling, receptive fields, and relative rod/cone inputs of horizontal cells in the tiger salamander retina. *J Comp Neurol*, 499(3), 422–431. <https://doi.org/10.1002/cne.21117>
- Zhang, D. Q., Stone, J. F., Zhou, T., Ohta, H., & McMahon, D. G. (2004). Characterization of genetically labeled catecholamine neurons in the mouse retina. *Neuroreport*, 15(11), 1761–1765. <https://doi.org/10.1097/01.wnr.0000135699.75775.41>
- Zhang, D. Q., Wong, K. Y., Sollars, P. J., Berson, D. M., Pickard, G. E., & McMahon, D. G. (2008). Intraretinal signaling by ganglion cell photoreceptors to dopaminergic amacrine neurons. *Proc Natl Acad Sci U S A*, 105(37), 14181–14186. <https://doi.org/10.1073/pnas.0803893105>
- Zhang, D. Q., Zhou, T. R., & McMahon, D. G. (2007). Functional heterogeneity of retinal dopaminergic neurons underlying their multiple roles in vision. *J Neurosci*, 27(3), 692–699. <https://doi.org/10.1523/jneurosci.4478-06.2007>
- Zhang, J., & Hu, Y. (2020). Comparing silicone oil-induced ocular hypertension with other inducible glaucoma models in mice. *Neural Regen Res*, 15(9), 1652–1653. <https://doi.org/10.4103/1673-5374.276330>
- Zhang, J., Jung, C. S., & Slaughter, M. M. (1997). Serial inhibitory synapses in retina. *Vis Neurosci*, 14(3), 553–563. <https://doi.org/10.1017/s0952523800012219>
- Zhang, J., & Wu, S. M. (2004). Connexin35/36 gap junction proteins are expressed in photoreceptors of the tiger salamander retina. *J Comp Neurol*, 470(1), 1–12. <https://doi.org/10.1002/cne.10967>
- Zhang, Q., Xue, J., Tang, J., Wu, S., Liu, Z., Wu, C., Liu, C., Liu, Y., Lin, J., Han, J., Liu, L., Chen, Y., Yang, J., Li, Z., Zhao, L., Wei, Y., Li, Y., & Zhuo, Y. (2024). Modulating

amacrine cell-derived dopamine signaling promotes optic nerve regeneration and preserves visual function. *Sci Adv*, 10(31), eado0866.

<https://doi.org/10.1126/sciadv.ado0866>

Zhao, M., Toma, K., Kinde, B., Li, L., Patel, A. K., Wu, K. Y., Lum, M. R., Tan, C., Hooper, J. E., Kriegstein, A. R., La Torre, A., Liao, Y. J., Welsbie, D. S., Hu, Y., Han, Y., & Duan, X. (2023). Osteopontin drives retinal ganglion cell resiliency in glaucomatous optic neuropathy. *Cell Rep*, 42(9), 113038. <https://doi.org/10.1016/j.celrep.2023.113038>

Zhao, X., Wong, K. Y., & Zhang, D. Q. (2017). Mapping physiological inputs from multiple photoreceptor systems to dopaminergic amacrine cells in the mouse retina. *Sci Rep*, 7(1), 7920. <https://doi.org/10.1038/s41598-017-08172-x>

Zhou, C., Robert, M. C., Kapoulea, V., Lei, F., Stagner, A. M., Jakobiec, F. A., Dohlman, C. H., & Paschalis, E. I. (2017). Sustained Subconjunctival Delivery of Infliximab Protects the Cornea and Retina Following Alkali Burn to the Eye. *Invest Ophthalmol Vis Sci*, 58(1), 96–105. <https://doi.org/10.1167/iovs.16-20339>