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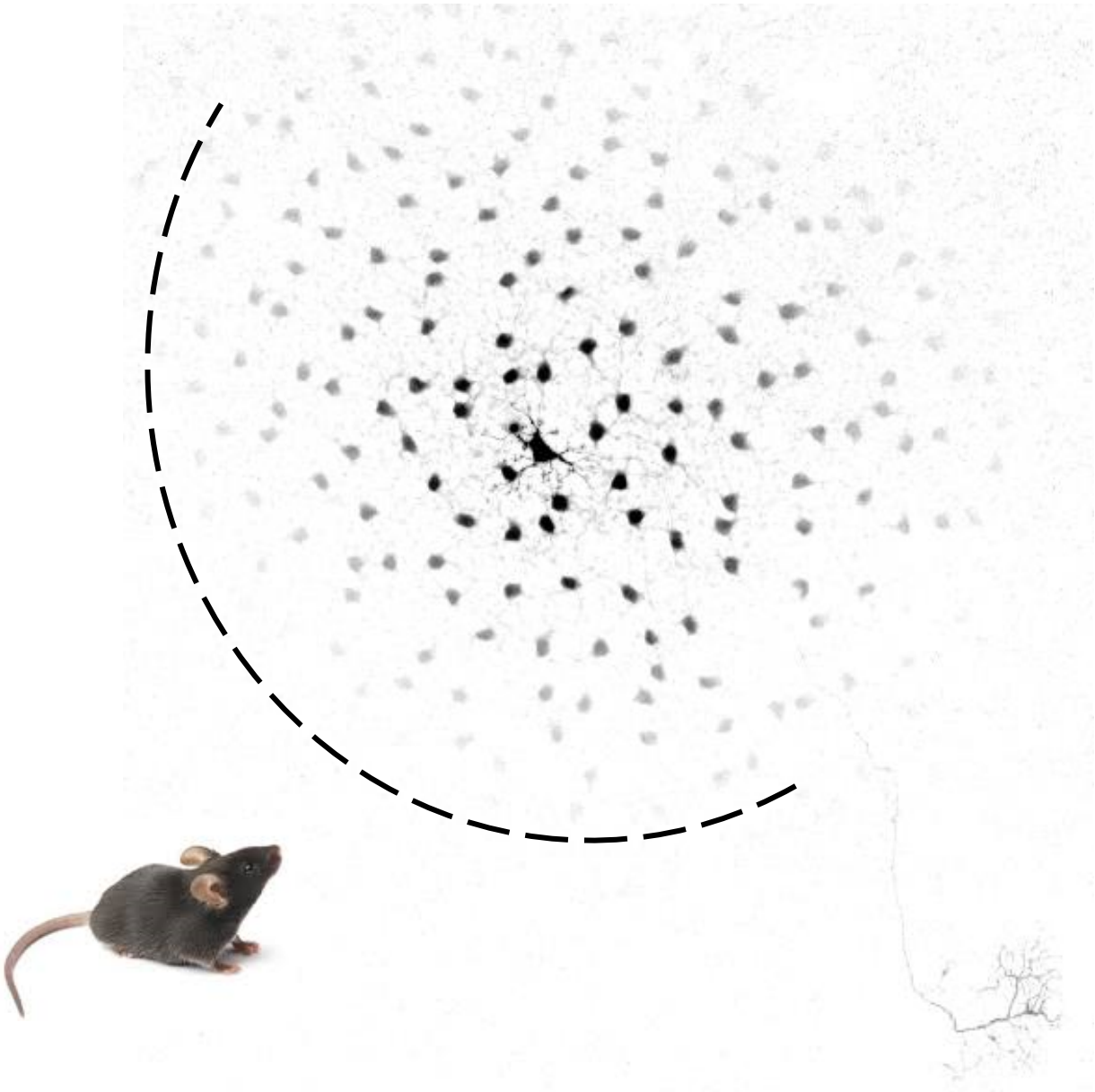
Morphometric analysis and synaptic contacts of horizontal cells in the mouse retina



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Morphometric analysis and synaptic contacts of horizontal cells in the mouse retina

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Table of contents

1. Summary

Vision is one of the primary sensory modalities in many species, including humans. It involves the ability to perceive and interpret visual information from the outside world and plays a fundamental role in how organisms interact with their environment. The process of vision is a complex interplay of physiological and neural processes that starts in photoreceptors with the absorption of photons by light-sensitive opsins in the outer segments of photoreceptors. Particularly in the mouse retina, rod photoreceptors express only a single opsin (rhodopsin). In contrast, cone photoreceptors express two types of opsins: M and S. Thus, rods and cones mediate scotopic and photopic vision, respectively.

The transmission of visual signals to the brain is a complex process involving substantial neural processing. The signal passes from photoreceptors to bipolar cells and subsequently from bipolar cells to ganglion cells, the retinal output neurons. This neural processing is significantly modulated by lateral inhibitory networks in the outer and inner retina by horizontal and amacrine cells, respectively. In this context, this dissertation focuses on horizontal cells.

Horizontal cells are laterally oriented interneurons strongly coupled to each other through gap junctions, forming extensive networks. In the mouse retina, horizontal cells are coupled through connexin 57 (Cx57). Due to the strong coupling, the receptive field of horizontal cells extends their dendritic field, allowing them to average the input from a wide array of photoreceptors. The commonly accepted understanding of the function of horizontal cells is to provide feedback to photoreceptors and feedforward signaling to bipolar cells, establishing, in this way, the antagonistic center-surround organization on downstream neurons through lateral inhibition. Thereby, horizontal cells contribute to contrast enhancement, color opponency, and light adaptation.

In the past, it was believed that the mouse retina was a homogeneous tissue, suggesting that different retinal types performed the same function regardless of their location within the retina. However, investigations over the past few decades have unveiled unexpected anatomical and physiological topographic variations across different cellular types characterized by different non-uniformity patterns. For example, research conducted by Warwick *et al.,* 2018 provides evidence that in the mouse retina, the light responses of a particular type of ganglion cell, the transient OFF-α retinal ganglion cell, differs across the dorsoventral axis. Thus, cells located in the ventral retina displayed transient responses to light decrements, whereas those in the

dorsal retina displayed sustained responses to light decrements. These findings strongly suggest that ganglion cells of the same type do not uniformly display identical light responses.

Consequently, cells of the same neuronal type may present variations in their distribution, morphology, and functional roles. The spatial arrangement of these variations enhances or encodes specific features of the external environment, highlighting that visual space is not uniformly sampled across the mouse retina. Consequently, it becomes essential to account for these topographical variations when conducting investigations related to visual processing. While topographic variations have been well-documented for various retinal cell types, such as bipolar and ganglion cells, less is known about topographic variations in horizontal cells. Several studies have highlighted disparities in the density of these cells within the mouse retina. Nonetheless, these investigations associated density with retinal eccentricity, focusing on the central and peripheral regions of the retina while overlooking the possibility of non-uniform variations in horizontal cell density across different retinal areas.

Thus, to investigate further potential topographic variations in horizontal cells, I conducted immunohistochemistry experiments, intracellular dye/tracer injections, and confocal microscopy on whole-mount preparations of retinas from wild-type mice. The aim was to get a deeper insight into the morphology of horizontal cells by analyzing their density, branching pattern, and termination points through a Sholl analysis. In addition, I investigated the coupling pattern of horizontal cells across the dorsoventral axes of the retina by injecting a tracer molecule that can pass through gap junctions.

In collaboration with Matteo Spinelli, the findings provided in this study revealed the existence of a gradient of Cx57 along the dorsoventral axes of the retina, where a higher expression of this connexin was found on the dorsal side and lower expression on the ventral side. Furthermore, the results provide evidence of variations in the distribution of horizontal cells within the retina, with lower and higher densities in the dorsal and ventral regions, respectively. Interestingly, the horizontal cell gradient appears to overlap with the S-opsin gradient. Notably, there is a horizontal cell hotspot in the ventral retina that might potentially overlap with the true S-cones. The subsequent morphometric analysis indicated differences in the dendritic field size of the horizontal cells across the dorsoventral axes of the retina, where horizontal cells located on the dorsal side were bigger than their counterparts located on the ventral side. Nevertheless, these cells do not differ from each other in a significant manner; instead, they adjust their size to maintain a consistent coverage factor throughout the entire retina. Similarly,

the Sholl analysis did not reveal striking differences between horizontal cells across the dorsoventral axes of the retina; indeed, their arborization pattern remained constant. Additionally, I found that the horizontal cell coupling pattern varies across the dorsoventral axes of the retina, likely influenced by the differential expression of Cx57. Thus, I found more horizontal cells strongly coupled on the dorsonasal side, covering a substantial area within the retina. Conversely, I found fewer coupled horizontal cells in the ventral retina, covering a smaller area. The varying coupling patterns observed between the dorsonasal and ventronasal regions suggest that differences in the number of coupled cells and the area they cover cannot be solely attributed to cell density. Instead, they point to distinct physiological regulations. Specifically, it appears to be an increased electrical coupling of horizontal cells on the dorsal side, leading to broader coverage of larger fields. Conversely, a reduced expression of Cx57 in horizontal cells on the ventral side results in fewer coupled cells covering smaller fields. Variations in the horizontal cell coupling pattern could potentially impact the global signaling these cells provide to downstream neurons, such as bipolar cells and ganglion cells, by shaping their receptive fields. Indeed, these results align with a recent study from Gupta *et al.,* 2023 that identified consistent differences in the organization of ganglion cell receptive fields along the dorsoventral axes of the retina. In the mentioned study, the authors found that ganglion cell receptive fields located in the ventral retina showed smaller center sizes and strong surround strength. In contrast, ganglion cell receptive fields exhibit bigger center sizes in the dorsal retina and less surround strength.

The outcomes of the present study demonstrate the presence of topographic variations in horizontal cells across the dorsoventral axes of the mouse retina. These variations likely contribute to modulate specific functions within the retina, such as spatial and temporal processing, which might be reflected in the responses of downstream neurons such as bipolar cells and ganglion cells.

2. Zusammenfassung

Das Sehen ist bei vielen Lebewesen, auch beim Menschen, eine der primären Sinnesmodalitäten. Es beinhaltet die Fähigkeit, visuelle Informationen aus der Außenwelt wahrzunehmen und zu interpretieren und spielt eine grundlegende Rolle bei der Interaktion von Organismen mit ihrer Umwelt. Der Sehvorgang ist ein komplexes Zusammenspiel physiologischer und neuronaler Prozesse, das in Photorezeptoren mit der Absorption von Photonen durch lichtempfindliche Opsine beginnt, die in den äußeren Segmenten der Photorezeptoren enthalten sind. Insbesondere in der Netzhaut von Mäusen exprimieren Stäbchen-Photorezeptoren nur ein einziges Opsin (Rhodopsin), während Zapfen-Photorezeptoren zwei Arten von Opsinen exprimieren: M und S. Somit vermitteln Stäbchen und Zapfen das skotopische bzw. photopische Sehen.

Die Übertragung visueller Signale an das Gehirn ist ein komplexer Prozess, der eine erhebliche neuronale Verarbeitung erfordert. Das Signal gelangt von den Photorezeptoren zu den Bipolarzellen und anschließend von den Bipolarzellen zu den Ganglienzellen, den Ausgangsneuronen der Netzhaut. Diese neuronale Verarbeitung wird maßgeblich durch laterale Hemmnetzwerke sowohl in der äußeren als auch in der inneren Netzhaut durch horizontale bzw. amakrine Zellen moduliert. In diesem Zusammenhang konzentriert sich diese Dissertation auf horizontale Zellen.

Horizontale Zellen sind seitlich ausgerichtete Interneurone, die über Gap Junctions stark miteinander verbunden sind und ausgedehnte Netzwerke bilden. In der Netzhaut der Maus sind horizontale Zellen über Connexin 57 (Cx57) gekoppelt. Aufgrund der starken Kopplung erweitert das Empfangsfeld horizontaler Zellen ihr dendritisches Feld, sodass sie den Input einer breiten Palette von Photorezeptoren mitteln können. Das allgemein akzeptierte Verständnis der Funktion horizontaler Zellen besteht darin, Feedback an Photorezeptoren und Feedforward-Signale an bipolare Zellen zu senden und auf diese Weise durch laterale Hemmung die antagonistische Zentrum-Umfeld-Organisation auf nachgeschalteten Neuronen zu etablieren. Dadurch tragen horizontale Zellen zur Kontrastverstärkung, Farbopposition und Lichtanpassung bei.

In der Vergangenheit glaubte man, dass die Netzhaut der Maus ein homogenes Gewebe sei, was darauf hindeutet, dass verschiedene Netzhauttypen unabhängig von ihrer Position innerhalb der Netzhaut die gleiche Funktion erfüllten. In den letzten Jahrzehnten durchgeführte Untersuchungen haben jedoch unerwartete anatomische und physiologische topografische

Variationen zwischen verschiedenen Zelltypen aufgedeckt, die durch unterschiedliche Muster der Ungleichmäßigkeit gekennzeichnet sind. Untersuchungen von Warwick *et al.*, aus dem Jahr 2018 liefern beispielsweise Belege dafür, dass in der Netzhaut der Maus die Lichtreaktionen eines bestimmten Typs von Ganglienzellen – der transienten OFF-α-Ganglienzellen der Netzhaut – über die dorsoventrale Achse hinweg unterschiedlich sind, also die lokalisierten Zellen in der ventralen Netzhaut zeigten vorübergehende Reaktionen auf Lichtabnahmen, während jene in der dorsalen Netzhaut anhaltende Reaktionen auf Lichtabnahmen zeigten. Diese Ergebnisse deuten stark darauf hin, dass Ganglienzellen desselben Typs nicht einheitlich identische Lichtreaktionen zeigen.

Folglich können Zellen desselben neuronalen Typs Unterschiede in ihrer Verteilung, Morphologie und sogar in ihren funktionellen Rollen aufweisen. Die räumliche Anordnung dieser Variationen verstärkt oder kodiert bestimmte Merkmale der äußeren Umgebung und verdeutlicht, dass der visuelle Raum nicht gleichmäßig über die Netzhaut der Maus abgetastet wird. Daher ist es wichtig, diese topografischen Unterschiede bei der Durchführung von Untersuchungen im Zusammenhang mit der visuellen Verarbeitung zu berücksichtigen. Während topografische Variationen für verschiedene Netzhautzelltypen wie Bipolarzellen und Ganglienzellen gut dokumentiert sind, ist über topografische Variationen in horizontalen Zellen weniger bekannt. Mehrere Studien haben Unterschiede in der Dichte dieser Zellen in der Netzhaut der Maus aufgezeigt. Dennoch wurde bei diesen Untersuchungen die Dichte mit der Exzentrizität der Netzhaut in Verbindung gebracht, wobei der Schwerpunkt auf den zentralen und peripheren Regionen der Netzhaut lag, während die Möglichkeit ungleichmäßiger Schwankungen der horizontalen Zelldichte in verschiedenen Netzhautbereichen außer Acht gelassen wurde.

Um weitere potenzielle topografische Variationen in horizontalen Zellen zu untersuchen, führte ich daher immunhistochemische Experimente, intrazelluläre Farbstoff-/Tracer-Injektionen und konfokale Mikroskopie an Ganzkörperpräparaten von Netzhäuten von Wildtyp-Mäusen durch. Ziel war es, einen tieferen Einblick in die Morphologie horizontaler Zellen zu erhalten, indem deren Dichte, Verzweigungsmuster und Endpunkte mithilfe einer Sholl-Analyse analysiert wurden. Darüber hinaus untersuchte ich das Kopplungsmuster horizontaler Zellen über die dorsoventralen Achsen der Netzhaut, indem ich ein Tracermolekül injizierte, das Gap Junctions passieren kann.

In Zusammenarbeit mit Matteo Spinelli zeigten die in dieser Studie bereitgestellten Ergebnisse die Existenz eines Gradienten von Cx57 entlang der dorsoventralen Achsen der Netzhaut, wobei auf der dorsalen Seite eine höhere Expression dieses Connexins und auf der ventralen Seite eine geringere Expression festgestellt wurde. Darüber hinaus liefern die Ergebnisse Hinweise auf Variationen in der Verteilung horizontaler Zellen innerhalb der Netzhaut mit geringerer bzw. höherer Dichte im dorsalen und ventralen Bereich. Interessanterweise scheint sich der horizontale Zellgradient mit dem S-Opsin-Gradienten zu überlappen und insbesondere gibt es einen horizontalen Zell-Hotspot in der ventralen Netzhaut, der möglicherweise mit den echten S-Zapfen überlappt. Die anschließende morphometrische Analyse zeigte Unterschiede in der Größe des dendritischen Feldes der horizontalen Zellen entlang der dorsoventralen Achsen der Netzhaut, wobei horizontale Zellen auf der dorsalen Seite größer waren als ihre Gegenstücke auf der ventralen Seite. Dennoch unterscheiden sich diese Zellen nicht wesentlich voneinander; vielmehr passen sie ihre Größe an, um einen gleichmäßigen Abdeckungsfaktor über die gesamte Netzhaut hinweg aufrechtzuerhalten. Ebenso ergab die Sholl-Analyse keine auffälligen Unterschiede zwischen horizontalen Zellen entlang der dorsoventralen Achsen der Netzhaut; tatsächlich blieb ihr Baumstrukturmuster konstant. Darüber hinaus fand ich heraus, dass das Kopplungsmuster der horizontalen Zellen entlang der dorsoventralen Achse der Netzhaut variiert, was wahrscheinlich durch die unterschiedliche Expression von Cx57 beeinflusst wird. Daher fand ich eine größere Anzahl horizontaler Zellen, die auf der dorsonasalen Seite stark gekoppelt waren und einen beträchtlichen Bereich innerhalb der Netzhaut abdeckten. Im Gegensatz dazu fand ich in der ventralen Netzhaut weniger gekoppelte horizontale Zellen, die einen kleineren Bereich abdecken. Die unterschiedlichen Kopplungsmuster, die zwischen den dorsonasalen und ventronasalen Regionen beobachtet werden, legen nahe, dass Unterschiede in der Anzahl der gekoppelten Zellen und der von ihnen bedeckten Fläche nicht ausschließlich auf die Zelldichte zurückzuführen sind. Sie weisen vielmehr auf unterschiedliche physiologische Regelungen hin. Konkret scheint es sich um eine verstärkte elektrische Kopplung horizontaler Zellen auf der Rückenseite zu handeln, die zu einer breiteren Abdeckung größerer Felder führt. Umgekehrt führt eine verringerte Expression von Cx57 in horizontalen Zellen auf der ventralen Seite dazu, dass weniger gekoppelte Zellen kleinere Felder abdecken. Variationen im horizontalen Zellkopplungsmuster könnten möglicherweise Auswirkungen auf die globale Signalübertragung haben, die diese Zellen an nachgeschaltete Neuronen wie Bipolarzellen und Ganglienzellen weitergeben, indem sie deren Empfangsfelder formen. Tatsächlich stehen diese Ergebnisse im Einklang mit einer aktuellen Studie von Gupta *et al.*, 2023, die konsistente Unterschiede in der Organisation der rezeptiven

Ganglienzellenfelder entlang der dorsoventralen Achsen der Netzhaut identifizierte. In der genannten Studie stellten die Autoren fest, dass die rezeptiven Ganglienzellenfelder in der ventralen Netzhaut kleinere Zentren und eine stärkere Umgebungsstärke aufwiesen, während die rezeptiven Felder der Ganglienzellen in der dorsalen Netzhaut größere Zentren und eine geringere Umgebungsstärke aufwiesen.

Die Ergebnisse der vorliegenden Studie zeigen das Vorhandensein topografischer Variationen in horizontalen Zellen entlang der dorsoventralen Achsen der Netzhaut der Maus. Diese Variationen tragen wahrscheinlich dazu bei, bestimmte Funktionen innerhalb der Netzhaut zu modulieren, wie z. B. die räumliche und zeitliche Verarbeitung, was sich in den Reaktionen nachgeschalteter Neuronen wie Bipolarzellen und Ganglienzellen widerspiegeln könnte.

3. Abbreviations

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

cGMP: Cyclic guanosine monophosphate

CO_2: Carbon dioxide

Cx: Connexin

C57Bl6/J: Black 6 wild-type mouse strain

DAPI: 4', 6-diamidino-2-phenylindole

GABA: γ-aminobutyric acid

GCL: Ganglion cell layer

GDP: Guanosin diphosphate

GluAs: AMPA-type glutamate receptors

Hz: Hertz

ILM: Inner limiting membrane

INL: Inner nuclear layer

IPL: Inner plexiform layer

KCL: Potassium chloride

kDA: Kilodalton

LUT: Lookup tables

mGluR6: Metabotropic glutamate receptor

µL: Microliter

mM: Milimolar

mm^2: Square millimeter

ms: millisecond

MΩ: Megaohm

nA: nano ampere

$NaHCO_3$: Sodium bicarbonate

NFL: Nerve fiber layer

O_2: Oxygen

OLM: Outer limiting membrane

ONL: Outer nuclear layer

OPL: Outer plexiform layer

PB: Phosphate buffer

PDE: Phosphodiesterase

PFA: Paraformaldehyde

SD: Standard deviation

TRP M1: Transient receptor potential cation channel subfamily M member 1

UV: Ultraviolet

4. Figures

5. Introduction

Vision is the sense that allows living organisms to perceive and interpret their surroundings by processing visible light. As one of the primary sensory modalities across several species, including humans, vision plays a fundamental role in how organisms interact with their environment. In the case of mice, vision serves multiple purposes related to their survival, including predator detection, locating food sources, navigating their surroundings, social interactions, reproduction, and learning (Gerl and Morris, 2008; Baden *et al.,* 2020).

Adapted to their nocturnal lifestyle, vision is essential for mice as prey animals for their overall survival. Compared to humans, mice possess relatively simple eyes, characterized by fewer color receptors (cones) and a higher prevalence of light-sensitive receptors (rods). This arrangement allows them to detect motion and variations in light levels, heightening their sensitivity to movement and helping them to detect predators and potential threats even in darkness (Gerl and Morris, 2008; Qiu *et al.,* 2021).

A deep understanding of the visual capabilities of mice holds significant importance across different scientific fields, particularly in neuroscience. Mice, frequently employed as model organisms, provide a valuable basis for investigating vision, perception, and the underlying neural processes associated with these functions.

Over the years, there was a prevailing belief that the mouse retina constituted a uniform tissue where all retinal types were alike, regardless of their specific retinal location (Dräger and Olsen, 1981; Jeon *et al.,* 1998). However, evidence from the last decade has contradicted this notion, demonstrating significant topographic variations in function (Wässle *et al.,* 2009; Bleckert *et al.,* 2014; Denman *et al.,* 2018; Warwick *et al.,* 2018; Szatko *et al.,*2020; Gupta *et al.,* 2023) and distribution (Hughes *et al.,* 2013; Bleckert *et al.,* 2014; Camerino *et al.,* 2020; Nadal-Nicolas *et al.,* 2020), among several retinal cell types, such as bipolar cells, and ganglion cells throughout the retina. Nevertheless, there is limited knowledge about topographic variations in horizontal cells. Given the role of these cells in shaping the receptive fields of photoreceptors and bipolar cells and thereby also ganglion cells (Thoreson and Mangel, 2012; Ströh *et al.,* 2018; Gaynes *et al.,* 2022), by providing feedback to photoreceptors and feedforward signaling to bipolar cells (Thoreson *et al.,* 2008; Kramer and Davenport, 2015; Chapot *et al.,* 2017), this dissertation aims to contribute to our understanding of potential topographic variations in horizontal cells across the mouse retina.

To achieve this, I conducted intracellular dye/tracer injections combined with immunohistochemistry experiments and confocal microscopy. I performed single horizontal

cell reconstructions to analyze the morphology and coupling pattern of horizontal cells across the dorsoventral axis of the retina. Subsequently, the following chapters will extensively explore scientific aspects of visual signal processing, primarily emphasizing horizontal cell function. The ultimate goal is to provide the reader with essential insights to comprehend the focal subject of this dissertation.

5.1 Retinal layers

The retina is the receptor organ of the visual system. It comprises a thin layer of tissue that covers the back of the eye and is responsible for detecting light and converting it into electrical signals that the brain can interpret as visual information. The retina comprises five distinct classes of neurons organized into three nuclear layers separated by two synaptic layers interconnected through electrical and chemical synapses. Each layer plays a specific role in visual processing, and they come together to form functional circuits designed to detect specific light variations and motion patterns (Masland, 2012). For a better understanding, a brief description of the different retinal layers is provided (Figure 1). The first retinal layer is the inner limiting membrane (ILM), which structurally constitutes the boundary between the vitreous and the retina, forming a diffusion barrier (Mahabadi and Khalili, 2023). The nerve fiber layer (NFL) constitutes the second innermost layer of the retina, and it is composed of axons from retinal ganglion cells responsible for gathering visual impulses originating from rods and cones. The ganglion cell layer (GCL) contains the ganglion cells and displaced amacrine cells; subsequently, within the inner plexiform layer (IPL), amacrine cells establish synaptic connections with bipolar cells and ganglion cells. The inner nuclear layer (INL) contains the cell bodies of amacrine cells, bipolar cells, and horizontal cells, while in the outer plexiform layer (OPL), the first synaptic layer, bipolar cells establish synaptic connections with photoreceptors (rods and cones) and horizontal cells. The outer nuclear layer (ONL) contains the cell bodies of rods and cones. The outer limiting membrane (OLM) is believed to preserve retinal structure by providing mechanical support (Omri *et al.,* 2010). The outermost part of the retina contains the outer and inner segments of rods and cones, where the phototransduction cascade begins. Finally, the retinal pigment epithelium provides nourishment and support to the retina and also, due to its melanin content, reduces damage to the retina caused by UV light (Tian *et al.,* 2021).

In a broad sense, all the different retinal classes of neurons can be distinguished by their morphology and synaptic connections. Overall, the anatomy of the retina represents a highly

specialized tissue where all the different cell types are intricately coordinated, working together to allow for efficient detection and transmission of visual information. Therefore, the retina is a sophisticated research model to investigate information processing within neural networks.

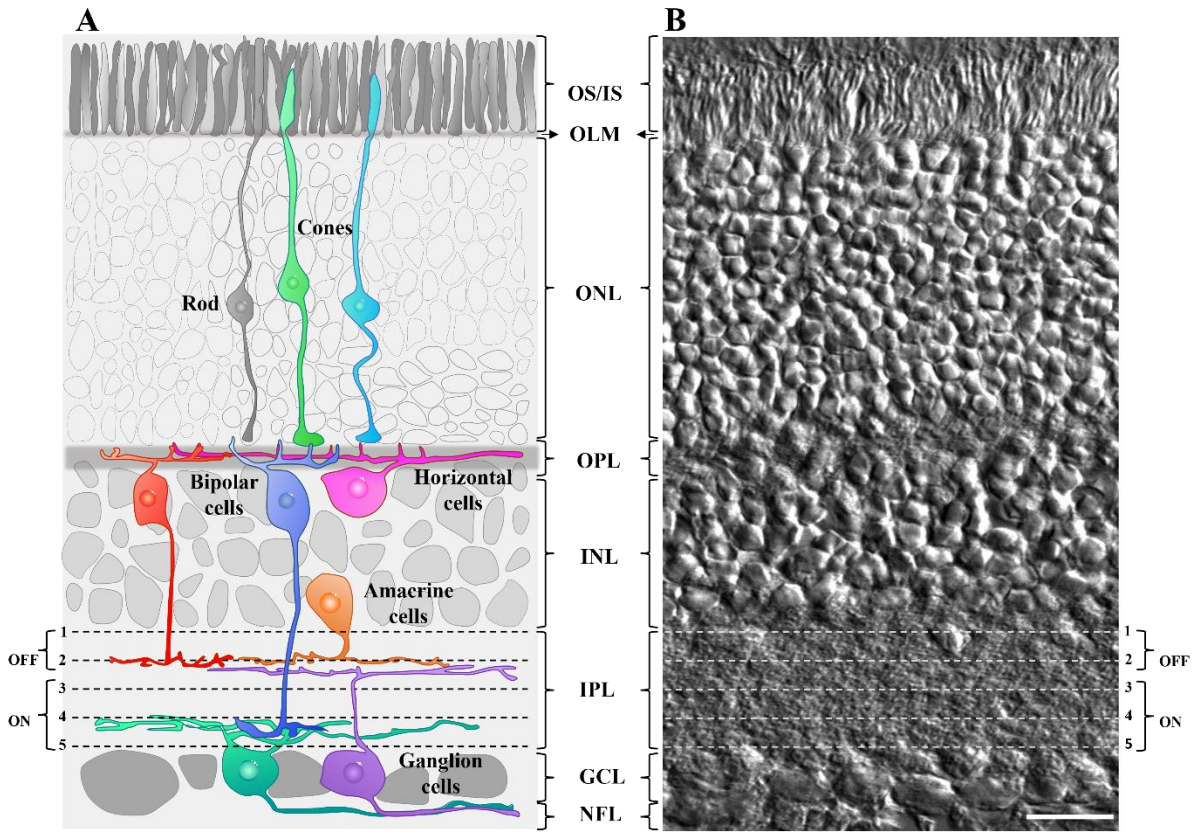


Figure 1| Schematic of the mouse retina. (A) The retina is vertically organized into several layers, each containing different classes of neurons. There are five major classes of retinal neurons: Photoreceptors (rods and cones), bipolar cells, horizontal cells, amacrine cells, and ganglion cells (left). (B) The right panel shows a transmission confocal microscopy image of a vertical retinal section of a mouse retina, where its laminar distribution is evident. Dashed lines within the IPL denote the ON/OFF ChAT bands, with the upper and lower parts representing these distinct bands, respectively. These ChaT bands serve as a landmark of the IPL. OS/IS: outer/inner segments of rods and cones, OLM: outer external limiting membrane ONL: outer nuclear layer, OPL: outer plexiform layer, INL: Inner nuclear layer, IPL: inner plexiform layer, GCL: ganglion cell layer, NFL: optic nerve fiber layer. Inner limiting membrane (ILM) and pigment epithelium (PE) are not shown. Scale bar: 20 µm. (A) created with vector schematics from Baden, T.; 2020. Available at http://github.com/BadenLab/Free_to_use_vector_graphics; CC by 4.0 creative License)

5.2 Phototransduction

Phototransduction is the process of converting light signals into electrical signals by photoreceptors. This cascade of biochemical reactions is essential for vision and occurs in rods and cones, which detect light and transmit visual information to the brain. The process begins when photons of light are absorbed by the visual pigments found within the outer segments of photoreceptors. In rods, the visual pigment is called rhodopsin, whereas cones possess different types of visual pigments, each sensitive to distinct wavelengths of light (i.e., different colors) (Lamb, 1995; Baylor *et al.*, 1987; Schnapf *et al.*, 1987; HarveyJr, 2001). Once a photon of light is absorbed, it triggers a conformational change in the chromophore 11-cis-retinal to all-trans-retinal within the visual pigment. This change, known as isomerization, is the key event that initiates the phototransduction cascade, leading to a hyperpolarization of the cell. Isomerization of 11-cis-retinal leads to a conformational change in the opsin molecule, which activates a protein called transducin. This G-protein typically exists in an inactive state while bound to GDP (guanosine diphosphate). When transducin becomes active, its α- subunit dissociates from the β- and γ- subunits. The α- subunit subsequently associates with the inhibitory subunit (γ) of phosphodiesterase (PDE), resulting in the activation of PDE. This activation catalyzes the hydrolysis of cyclic guanosine monophosphate (cGMP) (Arshavsky *et al.*, 2002; Luo *et al.*, 2009). In the dark state, cGMP levels are high, keeping ion channels in the photoreceptor cell membrane open. However, as cGMP levels decrease due to PDE activity, the ion channels in the photoreceptor cell membrane begin to close. This closure leads to the hyperpolarization of the cell, which reduces the release of the neurotransmitter glutamate from the photoreceptor cell onto bipolar cells (reviewed by Burns *et al.*, 2005), the second-order neurons in the visual pathway. Reduced glutamate release from photoreceptors subsequently activates bipolar cells in the retina. These bipolar cells transmit signals to ganglion cells, which in turn send signals to the brain via the optic nerve.

Subsequently, phototransduction deactivation assumes a critical role in preserving the sensitivity and accuracy of the visual system. After the cessation of light stimulation, all trans-retinal is reconstituted to 11-cis-retinal. As cGMP levels gradually increase due to reduced PDE activity, ion channels within the photoreceptor cell membrane begin to reopen, allowing photoreceptors to return to their resting membrane potential. The increase in cGMP levels also leads to an increase in glutamate release from photoreceptors (Burns, 2010). This neurotransmitter release resumes normal synaptic communication between photoreceptors and bipolar cells. Bipolar cells and ganglion cells in the retina, which receive signals from

photoreceptor cells, recover from their hyperpolarized state and resume their normal firing patterns. Phototransduction deactivation allows the visual system to adapt effectively to changing light conditions. When transitioning from a bright environment to a dark one, photoreceptors can quickly regain their sensitivity, whereas, in bright light, they become less sensitive. This intricate interplay between activation and deactivation ensures that the visual system can perceive a wide range of light intensities and effectively adapt to fluctuating lighting conditions (Detwiler and Gray-Keller, 1996; reviewed by Burns and Baylor, 2001). Consequently, the phototransduction cascade is a highly regulated and sensitive process that allows the detection of even small amounts of light. Its significance transcends both scotopic (low-light, rod-mediated) and photopic (bright-light, cone-mediated) vision, firmly establishing its pivotal role in shaping our visual perception.

5.3 Vertical signal transmission

In this process, visual information is transmitted vertically through all retinal layers, transmitting visual signals to the optic nerve and onward to the brain. This process involves several critical neuronal classes and synaptic connections within the retina. The process begins with photoreceptors converting light into electrical signals through the previously described phototransduction cascade. During the dark-adapted state, photoreceptors remain depolarized and constantly release the neurotransmitter glutamate. Conversely, in the light-adapted state, photoreceptors undergo hyperpolarization, resulting in a reduction of glutamate release. This glutamatergic signaling is transmitted to bipolar cells, categorized into two primary groups: ON bipolar cells and OFF bipolar cells. ON bipolar cells express metabotropic glutamate receptors, specifically mGluR6, coupled to G protein. When glutamate levels decrease, mGluR6 receptors initiate a signaling cascade that leads to the closure of cation channels (TRP M1) in the dendrites of ON bipolar cells. This channel closure causes a hyperpolarization of the ON bipolar cell (Nakajima *et al.*, 1993; Nawy, 1999; Morgans *et al.*, 2009; Koike *et al*., 2010). Thus, these cells are excited by light and inhibited by darkness. Subsequently, depolarized ON bipolar cells release glutamate, leading to an increased firing rate of ganglion cells that receive input from ON bipolar cells. These ganglion cells play a vital role within the ON pathway, transmitting signals related to increases in light intensity. In contrast, OFF bipolar cells express ionotropic glutamate receptors, specifically AMPA/kainate receptors, which are ion channels that open when glutamate levels increase. The binding of glutamate to AMPA/kainate receptors on OFF bipolar cells results in depolarization, the opposite of what

occurs in ON bipolar cells. Thus, OFF bipolar cells are inhibited by light and excited by darkness. Consequently, OFF bipolar cells transmit their signals to specific types of ganglion cells that belong to the OFF pathway. These ganglion cells convey information related to decreases in light intensity and respond to dark spots within the visual field.

The segregation of ON and OFF bipolar cell signals is propagated downstream by amacrine and ganglion cells. Signal transmission occurs within the inner plexiform layer (IPL), where ON and OFF bipolar cells ramify into separate strata (Figure 1) and shape the ganglion cell responses. The proximal section of the IPL serves as the OFF-signaling layer, enabling synaptic connections among OFF bipolar cells, amacrine cells, and ganglion cells. In contrast, in the distal IPL, ON bipolar cells, amacrine cells, and ganglion cells branch out to relay the ON signal. Recent investigations have unveiled many ganglion cell types within the retinas of mice and primates (Zhang *et al.*, 2012; Grünert and Martin, 2020; Kim *et al.*, 2021). These studies have primarily classified ganglion cells based on their distinctive branching patterns within the IPL, categorizing them into one of three main groups: ON, OFF, or ON-OFF ganglion cells. Ganglion cells extend their axons, collectively forming the optic nerve—a crucial pathway through which visual signals are conveyed in parallel pathways to the brain.

5.4 Modulation by horizontal cells

Horizontal cells are inhibitory neurons that extend their dendrites laterally within the outer plexiform layer of the retina. These cells are organized in a mosaic pattern, uniformly spaced concerning their counterparts (Masland, 2012). The regular spacing of the cell bodies and the alignment of the dendritic arbors at their edges represents the coverage factor. A coverage factor of 1 indicates no empty spaces between the dendritic arbor and no overlap between them (Masland, 2012). Consequently, the reported coverage factor for horizontal cells typically ranges between 5 and 7 (Reese *et al.*, 2005; Behrens *et al.*, 2022), suggesting that horizontal cells substantially overlap their dendrites with 5 to 7 neighboring cells.

The horizontal cell dendrites extend outward in a radial pattern within the OPL, receiving chemical synaptic input from photoreceptors and electrical synaptic input from other horizontal cells. The fine processes of horizontal cell dendrites, along with dendrites from ON bipolar cells, invaginate into the cone pedicle and rod terminals, forming a special arrangement known as the triad synapse (Villegas, 1964; Haverkamp *et al.*, 2000, reviewed by Burger *et al.*, 2021). In this arrangement, an ON bipolar cell dendrite is flanked by two horizontal cell dendrites. In

addition, OFF bipolar cell dendrites make flat contacts at the base of the photoreceptor terminals (Haverkamp *et al.,* 2000). Horizontal cells are essential in forming and maintaining the triad synapses. The ablation of horizontal cells in early postnatal mouse retinas prevents the invagination of rod bipolar cells into rods, disrupts the synaptic ribbon assembly, and reduces the expression of postsynaptic proteins at the dendritic tips of bipolar cells (Nemitz *et al.,* 2019). Hence, horizontal cells play a critical role in establishing synapse formation between photoreceptors and bipolar cells. These first synapses within the visual system serve as the primary relay points for information, fundamentally contributing to visual processing by accurately and precisely encoding information from the external world.

The light-induced response of a single photoreceptor is inhibited by the responses of surrounding photoreceptors in a process known as lateral inhibition. This mechanism establishes a receptive field arrangement characterized by a center-surround organization wherein light falling on the surroundings inhibits cell activity while light falling on the center excites the cell (Hartline *et al.,* 1956; Hartline and Ratliff, 1957). Thus, surrounds in the retina potentially increase sensitivity to edges in visual scenes through lateral inhibition (Marr and Hildreth, 1980). Horizontal cells stand as the primary components of lateral inhibition. Each horizontal cell receives chemical synaptic inputs from multiple rods and cones; in return, horizontal cells provide a feedback signal that modulates the neurotransmitter release of photoreceptors. Consequently, the interconnection between photoreceptors and horizontal cells is a reciprocal synapse. Horizontal cells provide local feedback to photoreceptors and also contribute to global processing (see discussion). In this scenario, horizontal cells are thought to act as a pathway for lateral information transmission within the retina (Dowling & Werblin, 1969). In this regard, these cells significantly contribute to organizing the receptive fields of downstream retinal neurons (Dowling & Werblin, 1969; Naka, 1972; Werblin, 1974; Byzov, 1975). Within the visual system, lateral inhibition improves the representation of contrast, enhancing the perception of edges. This process is essential for high-acuity daylight vision, primarily mediated by cones. However, recent findings suggest that horizontal cell feedback also extends to rods (Thoreson *et al.,* 2008).

When exposed to light, horizontal cells undergo sustained hyperpolarization by integrating inputs from photoreceptors. Due to the extensive electrical coupling of horizontal cells through gap junctions (see the following chapter), each cell receives photoreceptor input across a receptive field that extends beyond the horizontal cell dendritic field (Kaneko, 1971; Kaneko

and Stuart, 1984; Skrzypek. 1984; Dacheux and Miller, 1981; Dacheux and Raviola, 1982; Vaney, 1991; Bloomfield *et al.,* 1995).

The horizontal cell coupling is minimal under both bright (photopic) and dark (scotopic) light conditions, resulting in reduced receptive fields. In dim-scotopic light conditions, the horizontal cell coupling is maximal, leading to increased receptive fields. Thus, the horizontal cell coupling follows a triphasic pattern. Consequently, horizontal cell receptive fields might be directly controlled by the extent of electrical coupling (Xin and Bloomfield, 1999).

Horizontal cells receptive fields are reduced under bright light conditions (Ribelayga and Mangel, 2010), which is in line with studies demonstrating that the receptive fields of ganglion cells are also reduced under bright light conditions (Barlow *et al.,* 1957; Troy *et al.,* 1999). Several investigations, including one conducted by our working group (Stroh *et al.,* 2018), support the idea that horizontal cells contribute to the organization of receptive fields in downstream neurons like bipolar cells and ganglion cells. For example, in a research conducted by Chaya *et al.,* 2017 the authors created a horizontal cell-depleted mouse line in which electrophysiological analysis demonstrated that the center-surround receptive fields of ganglion cells are impaired and there is a reduction of the ON and OFF response diversities in ganglion cells. In a separate study, the authors demonstrated that chemogenetic perturbation of horizontal cells in the mouse retina resulted in either suppressed or enhanced responses of ganglion cells of the same polarity at different epochs of the response, even within the same ganglion cell (Drinnenberg *et al.,* 2018). Furthermore, Stroh *et al.,* 2018 developed a genetically modified mouse line where the light dependence of horizontal cell feedback was eliminated. By recording the light responses of transient OFF-α retinal ganglion cells in the retinas of the transgenic mouse, the authors found that the receptive fields of the recorded cells showed a significantly altered surround of their receptive fields. Moreover, in a recent study (Behrens *et al.*, 2022), researchers reconstructed the dendritic tree of five horizontal cells, along with the cone axon terminals and bipolar cell dendrites, to analyze their connectivity. The researchers identified short segments of increased diameter on the primary dendrites of horizontal cells, named “bulbs.” These bulbs represent GABAergic synapses that lack direct photoreceptor input but establish synaptic connections with bipolar cells and other horizontal cells. This outcome suggests the presence of two synaptic strata in the mouse retina (OPL), spatially segregating cone-specific feedback and feedforward signaling to bipolar cells. The authors propose that the GABAergic bulb synapses may relay global signals not only to other

horizontal cells but also to bipolar cells, potentially contributing to their center-surround antagonistic receptive fields (see discussion).

The findings mentioned above support the notion that horizontal cells contribute to the organization of the receptive fields of bipolar cells and ganglion cells. The influence of horizontal cells on the receptive fields of downstream neurons is believed to involve feedback from horizontal cells to cones and direct input from horizontal cells to bipolar cells. However, the mechanisms through which horizontal cells modulate this process have been widely debated (reviewed by Kramer and Davenport, 2015).

In summary, lateral inhibition, mediated by horizontal cells, is an important mechanism that optimizes the signal transmission from photoreceptors and shapes the receptive fields of bipolar cells and ganglion cells in the retina (Barnes *et al.,* 2020).

5.5 Electrical synapses

Neuronal communication occurs through different modalities, each characterized by unique spatial and temporal features. Nevertheless, the primary mechanism through which neurons communicate is via synapses, enabling the transmission of information across neural circuits within a timeframe ranging from milliseconds to seconds (Connors & Long, 2004). There are two primary types of synapses: chemical and electrical. The first relies on releasing neurotransmitters like glutamate, dopamine, or serotonin. In contrast, electrical synapses are mediated through intercellular channels known as gap junctions, which facilitate the direct flow of electrical currents between two adjacent neurons (Loewenstein, 1981; reviewed by Martin *et al.,* 2020). Since a part of this study focuses on the electrical synapses of horizontal cells, this chapter provides a compact introduction to gap junctions expressed by horizontal cells in the mouse retina, encompassing various aspects such as their structure, composition, function, and regulation.

Gap junctions are specialized intercellular channels that allow direct cytoplasmic exchange of small molecules, substrates, and metabolites between adjacent cells. Gap junctions are distinct from other membrane channels since they exist between two neighboring cells and have an apparent selectivity based principally on molecular size, allowing the movement of small molecules (~1 kDa) through passive diffusion (Saez *et al*., 2003; Nakase and Naus, 2004; Jiang and Gu, 2005; Zhou and Jiang, 2014; Bosone and Echeverria, 2016; Li *et al*., 2018). These

intercellular channels typically exist in clusters known as “gap junctional plaques” containing thousands of individual channels. Gap junctions comprise transmembrane proteins called connexins (Cx) that assemble into hexameric structures known as hemichannels or connexons. Each gap junction comprises two hemichannels from two neighboring cells forming a hydrophilic pore-like structure (reviewed by Bloomfield and Völgyi, 2009; Talukdar *et al*., 2022). As a result, the plasma membranes of adjacent cells join to form intercellular channels that enable the exchange of small molecules.

Gap junctions can be classified into several types based on the connexin molecular composition, which gives unique physiological properties to the channel, such as their permeability and gating; hence, the molecular composition of connexins ultimately dictates the role that a specific gap junction fulfills within the retinal circuitry. Hemichannels can comprise either a single connexin (homomeric) or multiple isoforms (heteromeric). Additionally, gap junctions may comprise two identical (homotypic) or two different (heterotypic) types of hemichannels. Likewise, gap junctions are highly specific, being able to establish connections between cells of the same type (homologous or homocellular) or different types (heterologous or heterocellular) (Söhl & Willecke, 2005; Volgyi *et al*., 2013a). Notably, not all combinations of connexins lead to functional connexons. Therefore, the incompatibility between specific pairs of connexons can result in communication barriers between groups of cells in contact. This, in turn, helps to prevent erroneous coupling between cells and to maintain their unique electrical characteristics (Kumar & Gilula, 1996). It has been described that connexins play crucial roles in a wide range of physiological processes, such as driving asynchronous firing, enhancing the signal-to-noise ratio, attenuating signals by shunting current and responsiveness of developing retina to light (reviewed by Vaughn and Haas, 2022).

Horizontal cells are interneurons that are strongly coupled through gap junctions. In the mouse retina, these cells are specifically coupled through Cx57, as demonstrated in a study where the researchers replaced the Cx57 coding region with a LacZ reporter gene to generate a Cx57-deficient mouse line. This deletion eliminated 99% of the coupling, affirming the pivotal role of Cx57 in horizontal cell connectivity (Hombach *et al.*, 2004). Moreover, it has been described that the axon terminals of horizontal cells in the mouse retina, in addition to Cx57, also express Cx50 (Dorgau *et al.*, 2015).

Conversely, gap junctional coupling is regulated by ambient light (Xin and Bloomfield, 1999) through several neuromodulators such as dopamine (Mangel and Dowling, 1985; Dong and

McReynolds, 1991; He *et al.,* 2000; Zhang *et al.,* 2011) retinoic acid (Weiler *et al.,*1999, 2000 Pottek and Weiler, 2000), and nitric oxide (Pottek *et al.,* 1997; Bloomfield & Völgyi, 2009; Daniels & Baldridge, 2011). Changes in Cx57 levels, phosphorylation, and gap junction structure are suggested mechanisms that drive light-dependent modulation of electrical coupling. Given the strong interconnection among horizontal cells via gap junctions, these cells efficiently collect light information across a large area of the retina, adjusting the gain of photoreceptors to different levels of ambient light. Synaptic gain refers to a change in cone calcium current per millivolt change in cone membrane potential. Therefore, horizontal cells provide lateral inhibition to cones and modulate the synaptic gain of photoreceptors (VanLeewen *et al.,* 2009).

In a different study, the authors aimed to characterize the receptive fields of horizontal cells (Shelley *et al.,* 2006) using the Cx57-deficient mouse line. The receptive fields of horizontal cells were significantly reduced in the transgenic mouse, accompanied by a substantial depolarization of dark resting potentials and reduced response amplitudes, suggesting an altered input resistance. The researchers also reaffirmed the unique role of Cx57 in horizontal cell coupling and physiology. Negative feedback to photoreceptors was intact in the Cx57-deficient mouse, ruling out a role for Cx57 in feedback to photoreceptors. However, the strong membrane depolarization of Cx57-deficient cells is likely to impact the feedback strength (Shelley *et al.,* 2006). This observation also suggests that Cx57 does not form hemichannels at the dendritic tips of horizontal cells (Janssen-Bienhold *et al.,* 2009). Consequently, horizontal cells play an essential role in adaptation by adjusting the cone sensitivity and shaping the receptive fields of downstream neurons, such as bipolar cells and ganglion cells. In addition, horizontal cells contribute to local and global processing, modulating the output to photoreceptors and organizing the center-surround receptive fields of bipolar cells and ganglion cells.

5.6 Gradients across the retina

In the past, it was commonly believed that the mouse retina was a homogeneous tissue (Dräger & Olsen- 1981; Jeon *et al.,* 1998), implying that all retinal types shared similar morphology and function regardless of their retinal location. This notion suggests that any given feature in the visual field is equally represented at any given location (Heukamp *et al.,* 2020). However, many investigations have unveiled regional variations in cellular distribution within the retina

across different species (Andrade-da-Costa *et al.,* 1989; Hughes *et al.,* 2013; Bleckert *et al.,* 2014; Camerino *et al.,* 2020; Nadal-Nicolas *et al.,* 2020). For example, the fovea and the visual streak are both specialized areas in the retina of the eye; however, they serve different purposes and have distinct characteristics. The fovea represents a compact area within the retina that typically lacks rod photoreceptors, while cone photoreceptors are highly concentrated and display elongated outer segments (Hendrickson, 2005; Polyak, 1941; Walls, 1942). The fovea is characterized by a lateral displacement of cells from the inner retinal layers, enabling a remarkably efficient packing of cones, resulting in minimal light scattering (Polyak, 1957). Consequently, the fovea is a small retinal area that enables high-acuity vision, and it is crucial for activities that require detailed vision, such as reading in humans and detecting small animals over long distances in birds of prey. Despite significant variation in foveal shape among different species, there are two fundamental types of fovea in vertebrates: Predatory fish, reptiles, and birds often have one or two convexiclivate (funnel-shaped) foveas in their retinas, whereas higher primates possess one concaviclivate (bowl-shaped) fovea (reviewed by Bringmann *et al.,* 2018).

On the other hand, the visual streak is found in animals that inhabit open fields, such as carnivores, kangaroos, foxes, chickens, rabbits, and pigs, among other species (Levick, 1967; Hughes, 1975; Schiviz *et al.,* 2008). This retinal specialization is an elongated region of high photoreceptor and ganglion cell density that often extends along the nasotemporal axis of the retina. For these species, the visual streak confers a panoramic view of the horizon that does not require eye or head movement, aiding in detecting prey and approaching predators (Hughes, 1977; Collin, 2008). Less frequently observed are vertical streaks spanning the dorsoventral axis of the retina. This adaptation is present in species whose visual field is characterized by vertically aligned elements, such as tree branches seen by the two-toed sloth (Andrade-da-Costa *et al.,* 1989) or water columns seen by species that migrate vertically, such as the teleost fish *Howella sherborni* (Collin & Partridge, 1996). While not as precise as the fovea regarding visual acuity, the visual streak facilitates peripheral vision and motion detection in these animals, allowing better detection of movement and objects in the periphery of their vision. In some cases, the fovea and the visual streak are specialized forms of an area centralis (Heukamp *et al.,* 2020).

In addition to these long-known regional specializations, more recent investigations demonstrate that topographic variations emerge in various forms, where different cellular types display uneven distributions, differ in their morphology, and exhibit variations in their physiology across the retina. For instance, while the general functional organization of the retina remains consistent across vertebrates, there are considerable differences in the arrangement of photoreceptors (Hunt and Peichl, 2014). In this regard, one of the best-known examples of gradients across the mouse retina involves the cone asymmetric expression of two opsin variants: M- and S- opsins, sensitive to medium and short wavelengths, respectively. The expression pattern of these opsins creates opposing gradients across the dorsoventral axes, resulting in a significant functional division of the mouse retina. Consequently, the mouse retina can be categorized into three major functional regions: 1) The dorsal retina, characterized by an enrichment of M-opsin expressing cones and minimal S- opsin co-expression; 2) the opsin transition zone, located at the horizon where the co-expressing ratio of M/S opsin increases and 3) The ventral retina, strongly dominated by S-opsin-expressing cones and minimal M-opsin co-expression. In addition, the ventral retina possesses a narrow region dominated by true S-cones, expressing only S-opsin (Baden *et al.,* 2013; Nicolas-Nadal *et al.,* 2020). Being a small animal, the mouse primarily senses its surroundings from ground level. This leads to a segmentation of the retinal image into the upper (ventral) and lower (dorsal) fields. As a result, the ventral retina faces the sky, while the dorsal retina is directed to the ground. This specialization allows the animal to effectively detect short-wavelength light from the sky in the upper field and ground-level visual cues, such as grassy fields, in the lower field (Szél *et al.,* 1992; Gouras & Ekesten, 2004; Osorio & Vorobyev, 2005; Baden *et al.,* 2013). The simultaneous expression of both opsins broadens the spectral range of individual cones (Röhlich *et al.,* 1994) and enhances perception in varying ambient light conditions (Chang *et al.,* 2013). The asymmetric distribution of opsins across the retina might play a pivotal role in evolutionary adaptation, enhancing the animal's visual field sensitivity and improving its chances of survival and mating. For instance, small animals constantly threatened by aerial predators might gain an advantage by adapting their spectral sensitivity towards short wavelengths in the upward-looking ventral retina (Szatko *et al.,* 2020). This sensitivity might enhance the contrast of objects against the sky, which is predominantly rich in shortwave components (Baden *et al.,* 2013). Conversely, long wavelengths in the downward-looking dorsal retina may be helpful for food search and orientation (Schiviz *et al.,* 2008).

On the other hand, in a research conducted by Camerino *et al.,* 2021, the authors mapped the distribution of six known OFF bipolar cells (1a, 1b, 2, 3a, 3b, and 4) concerning eccentricity and retinal domain. Their findings describe significant variation in the distribution of these cell types together with horizontal cells across the dorsoventral and central-peripheral axes of the retina. The functional effects of a skewed cell distribution are still unfolding; however, the distribution patterns of different cell types within retinal domains could potentially align with specialized functions that are adjusted to the visual demands (Warwick *et al*., 2018).

In addition, differences in the light responses of different retinal cell types have also been described. According to Warwick *et al.,* 2018, light responses of retinal ganglion cells of the same type (transient OFF-α retinal ganglion cells) gradually become more sustained across the dorsoventral axes of the retina, which is attributed to variances in their underlying circuits. The authors propose that networks underlying ganglion cell responses may change depending on retinal location, facilitating the optimized sampling of the visual image in the mouse retina.

Furthermore, a separate investigation discusses the organization of receptive fields of ganglion cells in response to the statistics of natural scenes. The study sought to understand how the structure of ganglion cells receptive fields and their surrounds vary across the visual field and the dorsoventral axes of the retina in mice. The findings of this study provide evidence that ganglion cell receptive fields are not uniform across the retina. Instead, they are organized to encode the panoramic structure and statistics of natural scenes efficiently. These insights carry implications for our understanding of sensory coding and retinal function (Gupta *et al.,* 2023).

In this context, as previously mentioned, horizontal cells modulate the feedback to photoreceptors and send a feedforward signaling to bipolar cells through lateral inhibition. This modulation is light-dependent and is potentially regulated by the extensive electrical coupling of horizontal cells through gap junctions. Consequently, it has been described that horizontal cells shape the receptive fields of downstream neurons, such as bipolar cells and ganglion cells (Ströh *et al*., 2018; Chaya *et al.,* 2017; Drinnenberg *et al.,*2018). Nevertheless, to this date, there is a lack of knowledge about topographical variations of horizontal cells across the mouse retina. Therefore, this dissertation aims to gain a deeper insight into potential variations of horizontal cells across the dorsoventral axis of the mouse retina, focusing primarily on their morphology and coupling pattern. This could shed light on how these cells contribute to visual processing and information transmission within the retina.

6. Aims

Different types of horizontal cells have been described across several species (reviewed by Peichl, 2010); however, in the mouse retina, only a single type of horizontal cell has been described: the axon-bearing horizontal cell, also known as B-type. In a recent study about the influence of horizontal cells on the light responses of transient OFF-α retinal ganglion cells (Ströh *et al.,* 2018), involving genetically modified mice in which the GluA2 and GluA4 subunits of AMPA receptors were selectively deleted in horizontal cells, revealed that the deletion of these receptor subunits in horizontal cells led to reduced spike rates and impaired temporal and contrast tuning in transient OFF-α retinal ganglion cells. In addition, this study shows that horizontal cells increase the dynamic range of retinal ganglion cells for contrast and temporal changes and contribute to the center-surround organization of the receptive fields of ganglion cells. On the other hand, another study analyzed the light responses of transient OFF-α retinal ganglion cells across the dorsoventral axes of the retina (Warwick *et al.,* 2018). This study shows that this transient OFF-α retinal ganglion cell type gradually changes its functional properties along this axis, with dorsal transient OFF-α retinal ganglion cells exhibiting longer-duration responses than ventral transient OFF-α retinal ganglion cells. These findings challenge the common belief that ganglion cells of the same type have uniform light responses across the retina. In addition, a recent study from Gupta *et al.,* 2023 discusses the organization and adaptation of receptive fields in retinal ganglion cells in response to the statistical structure of natural scenes, showing a systematic dorsoventral variation of the receptive field of ganglion cells, with a marked surround asymmetry at the visual horizon, regardless of the functional type. Given the role of horizontal cells shaping the receptive fields of ganglion cells, it becomes intriguing to explore potential topographic variations in the distribution of these cells in the mouse retina. Although several studies have delved into comprehending the functional and physiological aspects of horizontal cells, there is a lack of knowledge regarding topographic variations in the morphology and connectivity of the horizontal cells across the mouse retina. In consequence, the aims of this study are:

6.1 To gain a more comprehensive and in-depth understanding of the morphology of horizontal cells within the mouse retina

Several studies have focused on the morphology of horizontal cells across species (Boycott *et al.,* 1978; Boycott, 1988; Peichl & Gonzalez-Soriano, 1994; Linberg *et al.,* 1996; Hack & Peichl, 1999; Reese *et al.,* 2005; Raven *et al*., 2007; Raven *et al.,* 2008; Poché *et al.,* 2008). Nonetheless, these investigations did not account for retinal location, leading to a limited understanding of potential morphometric differences along the dorsoventral axes of the mouse retina. To get a deeper insight, this study aimed to:

1. Develop a well-designed and standardized dissection protocol that allows to keep track of retinal location.
2. Perform injections of intracellular dye into horizontal cells across the dorsoventral axes of the retina, resulting in labeled cells with visible morphology.
3. Reconstruct the skeletons of the injected horizontal cells using the Amira software to subsequently make a Sholl analysis that will provide quantitative data on the branching pattern, dendritic area, and termination points of the reconstructed horizontal cells, thereby revealing the horizontal cell dendritic complexity and potential variations across the retina.
4. Trace back the original location of the injected cells by reconstructing the retina into its spherical shape. This will help to understand how the morphology of horizontal cells varies across the dorsoventral axes.
5. Compare the morphology of the injected cells across the dorsoventral axes of the retina to identify potential morphometric differences between them.

6.2 To investigate the connectivity of horizontal cells by analyzing their coupling patterns across the dorsoventral axes of the retina

Horizontal cells are interconnected through gap junctions, specifically through Cx57 (Hombach *et al.,* 2004), forming an extensive network that provides feedback to photoreceptors and feedforward signaling to bipolar cells. Due to this extensive electrical coupling, the receptive field of horizontal cells extends beyond their dendritic field (Kaneko, 1971; Kaneko & Stuart, 1984; Skrzypek. 1984; Dacheux & Miller, 1981; Dacheux & Raviola, 1982; Vaney, 1991; Bloomfield *et al.,* 1995). Some studies demonstrate that horizontal cells contribute to the center-surround organization of ganglion cells (Warwick *et al.,* 2018; Ströh *et al.,* 2018). Therefore, potential variations in the coupling pattern of horizontal cells may potentially have

an impact on the center-surround organization of ganglion cells. Some studies have focused on the coupling pattern of horizontal cells in different species (Kaneko and Stuart, 1984; Tornqvist *et al.*, 1988; Skrzypek, 1984; Vaney, 1991,1993; Baldridge *et al.*, 1991; Bloomfield *et al.*, 1995; He *et al.*, 2000; Ribelayga and Mangel, 2003, 2010), however, to date, there is no evidence about disparities in the coupling pattern across the dorsoventral axes of mouse the retina. Therefore, this study aimed to:

1. Perform intracellular injections of neurobiotin into horizontal cells located in the dorsoventral axes of the retina. This will reveal the coupled counterparts of the injected cell.
2. Provide constant conditions and strictly controlled diffusion times to minimize experimental variations and contribute to the reliability and accuracy of the results.
3. Analyse and compare the coupling pattern between the dorsoventral axes of the retina to determine potential variations in electrical coupling, which might reflect functional adaptations in visual processing.

Overall, the expected outcomes will provide a comprehensive understanding of the spatial distribution, connectivity, and morphology of horizontal cells within the mouse retina. This knowledge could be crucial for understanding how horizontal cells contribute to visual processing and how they adapt to the specific functional demands of different retinal regions.

7. Methods

7.1 Animals and retinal preparation

All the experiments performed in this study were approved by the local animal care committee (*Niedersaechsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit*) in compliance with the guidelines for the welfare of experimental animals issued by the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the laws of the Federal Government of Germany (Tierschutzgesetz; BGBl. I S. 1206, 1313 and BGBl. I S. 1934). A wild-type mouse line (C57BL6/J) between 14-15 weeks old was used in these experiments. Mice (both sexes) were maintained under a 12-hour light/dark cycle and underwent dark adaptation for at least 1-2 hours before the experiment. Subsequently, the experiment was performed in complete darkness using infrared night goggles (No. G18597, Gutzeit-Gmbh). Mice were euthanized through carbon dioxide (CO_2) inhalation followed by cervical dislocation. The eyes were enucleated and dissected in Ames medium (Sigma/Biomol) supplemented with ~ 23 mM of sodium bicarbonate ($NaHCO_3$) and carbogen (95% O_2; 5%

CO_2) at ~ 30-32°C; pH 7.4. The cornea, iris, lens, and vitreous body were carefully removed. Five cuts were placed within the retina: two horizontal cuts (following the choroid fissure), two vertical cuts, and one final cut in the dorsotemporal side as a reference cut were made to be able to identify the retinal orientation by separating all retinal axes. Subsequently, the sclera was gently removed, and the retina was incubated in Ames medium containing 20 - 30 µL of DAPI ([1mg/mL]; Biomol) for 60 min to label cell nuclei. Finally, the retina was mounted ganglion cell side up on black nitrocellulose membranes (0.8 µm, MF Millipore) on a bath chamber and placed at the injection set-up with a constant flow of Ames medium for 10-15 min before intracellular dye injection.

7.2 Intracellular dye and neurobiotin injection

To analyze the horizontal cell morphology, borosilicate glass electrodes were pulled with a micropipette puller model P-97 (Sutter Instrument CO) to get sharp electrodes with resistance between 100 – 200 MΩ. The electrodes were filled with 2 µL of 5 mM of Alexa Fluor 588 hydrazide (Invitrogen, diluted in 200 mM KCL) and backfilled with 10 µL of 200 mM KCL. Pre-incubation of the retina with a nuclear dye (DAPI, described above) helped to identify horizontal cell somata based on retinal depth and size (~ 10 – 12 µm in diameter); the cells were targeted across all retinal axes using a fluorescent lamp (Mercury short arc HBO, OSRAM). Once a horizontal cell was found, the dye was ionthoporezed under visual control using -0.5 nA square pulses of 500 ms at 1 Hz for 3 minutes using an SEC-05LX amplifier (npi electronic GmbH). Note that a negative current was applied since the dye is positively charged. Subsequently, after injecting at least 1-2 cells, the retina was fixed in 2% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB) for 20 minutes (see below). From around 100 injected horizontal cells, 69 cells were used for analysis. The rest of the cells were dismissed due to co-injections or disrupted morphology. A total of 20 retinas (left and right) from 18 mice (both genders) were used for the horizontal cell morphology analysis. Similarly, to analyze the horizontal cell coupling, a tracer injection using 4% neurobiotin (SP1120, Biozol) diluted in 0.1 M Tris buffer (pH 7.3) was performed. Neurobiotin is a small molecule that can diffuse through gap junctions (Horikawa & Armstrong, 1988; Vaney, 1991). Thus, all the coupled horizontal cells can be further revealed. The injection was performed using the same electrodes described above, filled with 3 µL of a mixture of 4% neurobiotin and five mM Alexa Fluor 568 Hydrazide (1:1). Horizontal cells from peripheral dorsonasal and ventronasal sides of the retina were targeted. Once the cell was impaled, the dye was ionthoporezed as described above for 1-

2 minutes. After checking the quality of the injection (without leakage and undisrupted morphology of the cell), the current was reversed to inject neurobiotin using +0.5 nA square pulses of 500 ms at 1 Hz for 5 min and let it diffuse strictly for 10 min before fixation (see below). Note that a positive current was applied since neurobiotin is negatively charged. It is worth noting the difficulty of the tracing experiments; to provide constant conditions and due to the strictly controlled tracer injection and diffusion times, it was only possible to inject one horizontal cell per retina. Only 14 out of 43 retinas showed horizontal cell coupling. All of these retinas were used to compare the coupling of horizontal cells between dorsonasal and ventronasal retina.

7.3 Immunohistochemistry

After injection, the whole-mounted retinas [laying on black nitrocellulose membranes (0.8 µm, MF Millipore)] were fixed in 2% PFA diluted in 0.1 M PB for 20 min and washed afterward for 3 x 10 minutes in PB. To analyze the horizontal cell coupling, the retinas were incubated overnight at 4°C with Alexa Fluor 568 conjugated to streptavidin (1:250, Invitrogen), diluted in incubation buffer (10% donkey serum, 0.3% Triton X_{100}, 0.1M PB). Following incubation, the retinas were washed for 3 x 10 minutes with 0.1 M PB. Then, they were carefully positioned onto a slide layered with three strips of electrical tape. A hole precisely matching the size of the nitrocellulose membrane carrying the retinas was manually crafted at the center. Subsequently, ~ 30-50 µL of Vectashield (Vector Laboratories) was added as the mounting medium, and the tissue was securely sealed with a coverslip and nail polish. This mounting technique was designed to prevent tissue movement during image acquisition at the confocal laser scanning microscope. On the other hand, since the injected dye was enough to visualize the morphology of the horizontal cells, it was not necessary to perform any additional labeling. Therefore, after fixation and washing, the retinas were immediately mounted as previously described and kept at 4ºC until image acquisition.

7.4 Confocal microscopy acquisition and whole-mount retina reconstruction

An overview of the whole-mounted retinas was scanned at the Leica DM6 B upright microscope with the HCX PL Fluotar 20X/0.5 PH2 objective to subsequently trace back the exact localization of the injected horizontal cell within the retina. For this, the outline of the retina, temporal side, optic disc, and injected horizontal cells were marked using the "cell counter" plug-in in Fiji/Image J. The markers were saved as an "XML" file easily recognizable by the R package Retistruct (Sterratt *et al*., 2013), which was used to reconstruct the whole-mounted retinas into its half-spherical shape. All retinas were aligned horizontally following the choroid fissure cuts, and the orientation was identified following the reference cut at the dorsotemporal side. Retina alignment and reconstruction were done by Christoph Block. For the morphological analysis, stack images of all 69 cells were acquired using a confocal laser microscope (Leica TCS SP8) with the HC PL APO CS2 40X/1.3 and 63X/1.4 oil objectives. To determine the area of the dendritic field, the dendritic tree and the soma of each cell were manually marked with the "cell counter" plug-in on a maximum intensity projection image of each horizontal cell stack. Subsequently, using these markers, a convex hull was adjusted to each dendritic tree independently in Matlab. All horizontal cells were properly scaled based on the stack format size (micrometers). Convex hull adjustment and scaling of the cells were done by Christoph Block. For the coupling analysis, stack images of the coupled horizontal cells were acquired with the same confocal laser microscope mentioned above, using the HC PL APO CS2 20X/0.70 objective and the "LAS X Navigator" to scan the whole retinal area covered by the coupled horizontal cells. These cells were manually counted on a maximum intensity projection image using the ICA LUT on Fiji/Image J. To determine the area covered by the coupled horizontal cells, the outermost somata were manually marked, and subsequently, a convex hull was adjusted in Fiji/ImageJ. All 69 injected cells were plotted onto the horizontal cell density map for further analysis (work done by Christoph Block).

7.5 Horizontal cell reconstruction

The skeletons of 32 horizontal cells from different retinal areas were reconstructed with the Thermo Scientific Amira software. From these, nine cells were excluded by a second observer due to co-injections or interference from blood vessels, which hindered accurate interpretation. A Sholl analysis (Sholl, 1953) was conducted in Matlab to analyze the arborization of the remaining 23 cells, randomly distributed throughout the retina. All these cells were traced back to their original position within the retina and plotted all together in Figure 9. The primary

objective of this analysis was to examine the number of intersections of dendritic structures (representing the branching pattern) and the number of termination points (indicating dendritic tips) in concentric circles separated by 7.5 µm. The branching pattern was determined by dividing the number of intersections by the area of the circle. Similarly, the number of dendritic tips was calculated by dividing the number of termination points (within the annulus) by the area of the annulus. The sholl analysis and plotting of the cells into the heatmap were made by Christoph Block.

7.6 Statistical analysis

A Mann-Whitney test (GraphPad Prism 7) was performed to test for statistical significance ($p<0.05$) between the number of coupled cells and the area covered by them along the dorsoventral axis of the retina. Quantitative data was collected from 14 retinas, seven from each dorsal and ventral side. These values are represented in Figure 6 using the median as a means of comparison.

8. Results

8.1 Development of an experimental protocol

Recent publications (Stabio *et al.,* 2018; Sondereker *et al.,* 2018) emphasize the importance of considering retinal location when conducting experiments and analyzing data. Since different research groups employ different anatomical markers to identify retinal orientation, inconsistencies in the reliability and accuracy of these landmarks may give rise to discrepancies when identifying and reporting retinal topography. This, in turn, creates challenges when attempting to compare data across different studies. To prevent such discrepancies and reliably achieve the objectives outlined, an experimental protocol with a critical emphasis on keeping track of the retinal orientation was developed (Figure 2A-C).

Following the enucleation of the mouse eye (Figure 2A, A) and the subsequent removal of the cornea, iris, and lens, the retinal axes were identified by tracing the choroid fissure from a front view of the eye. The dorsal and ventral sides were distinguished by their positioning above and below the choroid fissure, respectively, flanked by the nasal and temporal sides (Figure 2A, B). Five cuts were strategically placed to separate the retinal axes to maintain the proper retinal orientation, as illustrated in Figure 2A, C. Following this, the sclera was carefully removed,

and the retina was incubated in Ames medium containing DAPI for an hour (see Methods). This step allowed for the visualization of horizontal cell somata. Subsequently, the retina was mounted in a bath chamber and placed in the setup with a continuous perfusion of Ames medium (Figure 2A, D). The temperature and pH of the solution were regularly monitored to ensure consistent experimental conditions. After a 10 – 15-minute period for the retina to acclimate to the environment, the intracellular dye injection was performed. The initial stage of the experimental protocol outlined above was followed to analyze both the morphology and coupling of horizontal cells. The experiments were conducted in complete darkness, facilitated by using infrared night goggles.

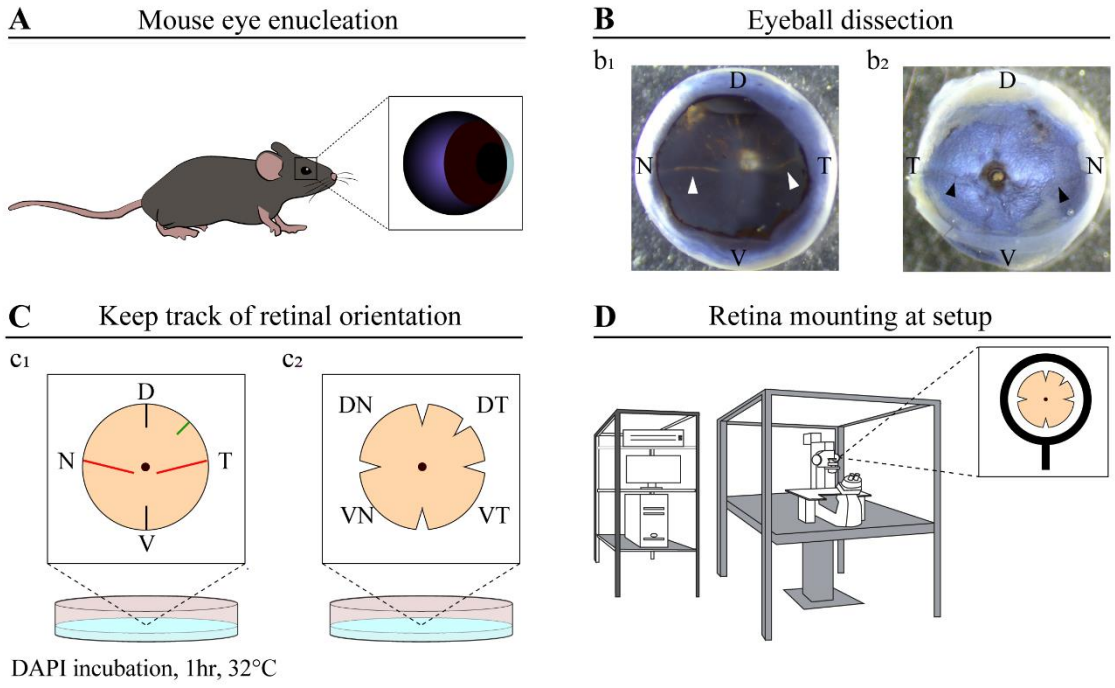


Figure 2A| Scheme illustrating the initial stage of the experimental protocol used in this study. A. After sacrificing the mouse, the left and right eyes were carefully enucleated and kept in separate Petri dishes. B. The eyeball dissection was performed in Ames medium. b_1.Front view of the mouse (right) eye after removing the cornea, iris, and lens. The retinal axes were identified following the choroid fissure (white arrowheads). D (dorsal), V (ventral), N (nasal), T (temporal). b_2. Bottom view of the mouse eye points to the choroid fissure (black arrowheads), used as a demarcation line to identify the dorsal and ventral sides of the retina. C. One of the key steps in this study was to keep track of the retinal orientation, for that (c_1), two cuts were made following the choroid fissure (red lines), two cuts were made vertically (black lines), and finally, a reference cut (green line) at the dorsotemporal side was made to identify the retinal orientation. Thus, all retinal axes were separated as dorsonasal (DN), dorsotemporal (DT), ventronasal (VN), and ventrotemporal (VT), as shown in c_2. The retinas were incubated for one hour with DAPI to label horizontal cell somata. D. The retina was mounted on a bath chamber at the setup with constant exchange of Ames medium. The temperature and pH were constantly measured to provide constant conditions for the experiment. For simplicity, the right retina is illustrated in all the figures of this dissertation.

To analyze the morphology, horizontal cells within all retinal axes were targeted and subsequently injected with Alexa 568 hydrazide (Figure 2B, A). After injecting at least 1-2 cells, the retina was fixed in 2% PFA for 20 minutes and thoroughly washed with PB 0.1M (Figure 2B, B). Since no additional labeling was necessary to analyze the morphology of horizontal cells, the retinas were mounted (see Methods) and kept at 4ºC until image acquisition. Later, an overview of the retinas was scanned to reconstruct them into their spherical form. For this, using the cell counter function in Fiji/ImageJ, the outline of the retina was demarcated along with the optic disk, the temporal side, and the individually injected cells (Figure 2B, D). Subsequently, an "XML" file was generated containing the coordinates of the mentioned markers, facilitating the reconstruction of the retina into its spherical form using Matlab and the R package Retistruct, which can be used to investigate the organization of the adult mouse visual system (Sterratt *et al*.,2013). The reconstructed retinas were aligned by placing a horizontal line in the middle of the sphere (Figure 2B, H) to superimpose them to identify and map the injected cells on a heatmap (Figure 2B, I). In total, 34 retinas were reconstructed: 20 retinas, including 69 injected horizontal cells, to analyze their morphology and 14 retinas injected with neurobiotin to analyze the coupling pattern. Retina reconstructions were done by Christoph Block (Figure 2B, H-I).

For the morphometric analysis, each injected horizontal cell was scanned in high resolution using a confocal microscope. The cell counter plugin in Fiji/ImageJ was employed on a maximum intensity projection of the scanned cells to mark the outermost dendrites and the soma as a center reference. A convex hull was then fitted to the dendritic tree to determine the dendritic field area of each of the 69 injected horizontal cells (Figure 2B, E). Of these, the skeletons of 32 horizontal cells randomly located within the retina were reconstructed using the Amira software (Figure 2B, F). As horizontal cells are very large and heavily branched (Figure 3A, B), the reconstruction of each cell took between 12-15 hours. Subsequently, to ensure a more precise interpretation of the results, nine cells were excluded by a second observer due to co-injected bipolar or Müller cells or interference with blood vessels. The final set comprised 23 reconstructed cells, which were plotted in Matlab for a subsequent Sholl analysis (Figure 2B, G) to determine the branching pattern and the number of intersections (dendritic pattern).

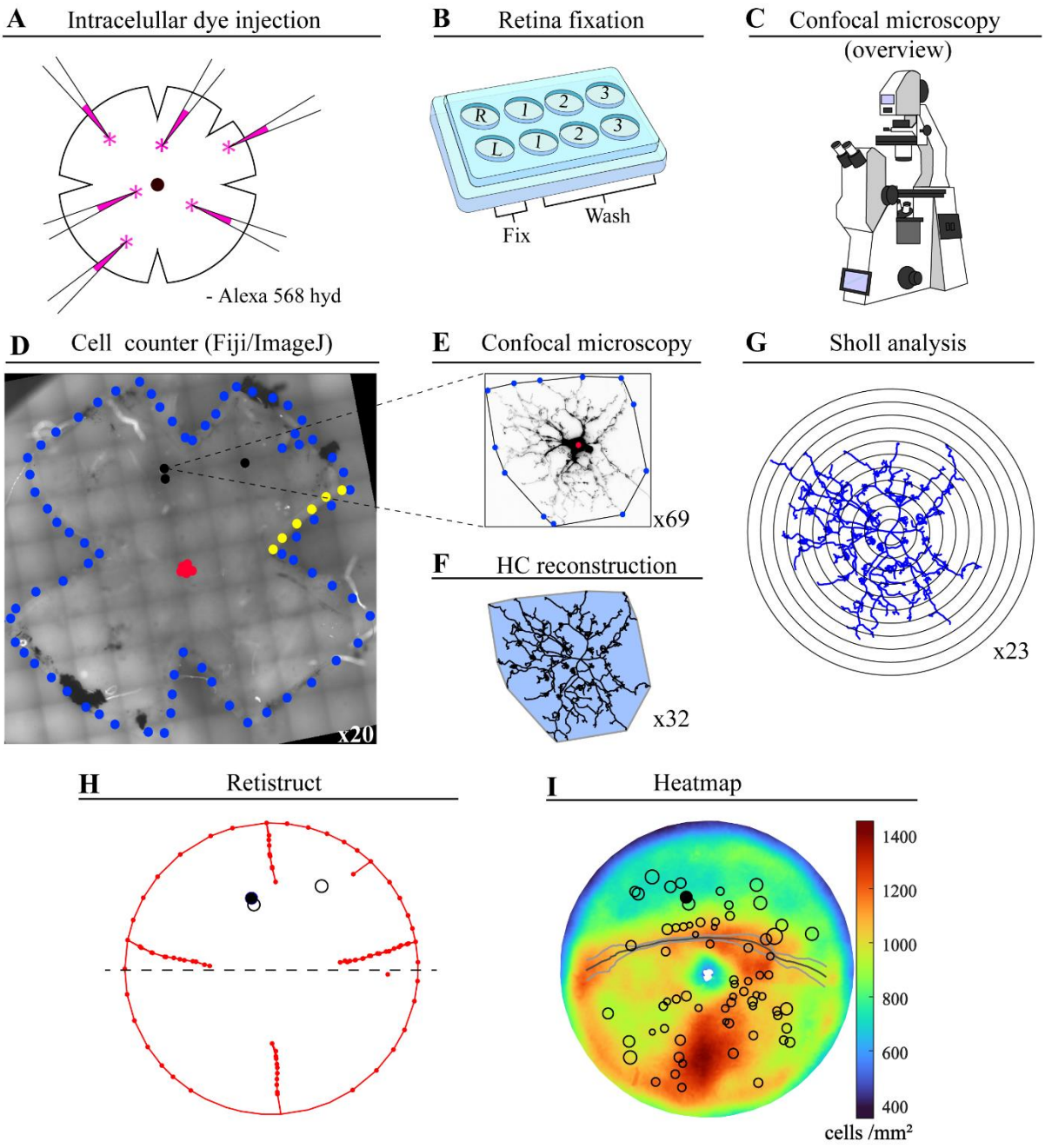


Figure 2B| Scheme illustrating the experimental protocol employed to analyze the morphology of horizontal cells. A. Intracellular dye injections with Alexa 568 hydrazide within different regions of the retina. B. After intracellular dye injection, the retina was immediately fixed in 2% PFA, washed three times with 0.1 M PB, and immediately mounted with Vectashield for further confocal scanning. C. An overview of the retina (shown in D) was scanned to subsequently reconstruct the retina into its original spherical shape. D. The overview of the retinas was used to reconstruct the retina in its original rounded shape. For that, the cell counter plugin from Fiji/ImageJ was used to mark the outline of the retina (blue), the optic disc (red), the temporal side (yellow), and the injected horizontal cells (black). For the morphology analysis, 20 retinas were scanned, each containing at least one injected horizontal cell. In the example, three cells (black dots) were injected and used for analysis, and one of them was reconstructed. It is worth mentioning that the outline of the retina does not perfectly match with the marker (blue dots). This is due to the presence of folds in some regions of the retina; as a

result, the marker was positioned at a location corresponding to the "unfolded" portion of the retina. Dot sizes were intentionally increased to facilitate visualization. E. Maximum intensity projection of the injected horizontal cell depicted in D. The cell counter plugin from Fiji was used to mark the outermost tips of the dendrites (blue dots) and the soma (red dot) as a center reference. A convex hull, the area enclosed within a convex polygon, was adjusted by connecting the outermost tips of the dendrites to determine the dendritic area. F. Horizontal cell reconstruction of the cell shown in E. The blue filling represents the dendritic area enclosed by a convex hull. 32 horizontal cells were reconstructed; however, nine cells were discarded by a second observer due to co-injections or interference with blood vessels. G. 23 reconstructed cells were plotted in Matlab, and subsequently, a Sholl analysis was performed to determine the branching pattern and the number of intersections (dendritic tips). H. Illustrates the reconstructed retina shown in D and the position of the three injected horizontal cells. The black dot represents the reconstructed horizontal cell shown in E. I. Illustrates a horizontal cell density map, including the position of all 69 injected horizontal cells from 20 retinas. The open circles represent the dendritic area of the injected cells. Retina reconstructions were done by Christoph Block (Figure 2C, H-I).

For coupling experiments, I restricted my injections to horizontal cell somata at the dorsonasal and ventronasal peripheral sides of the retina (Figure 2C, A). Once a horizontal cell soma was identified, Alexa 568 hydrazide was ionthoporezed to assess the injection quality. Horizontal cells displaying disrupted morphology or notable dye leakage were excluded, and the injection was stopped. Once a horizontal cell was successfully injected, neurobiotin, a small tracer molecule, was ionthoporezed into the cell for 5 minutes. Subsequently, the tracer was allowed to diffuse for 10 minutes. After that, the retina was immediately fixed in 2% PFA for 20 minutes and thoroughly washed with PB 0.1M. It is important to emphasize that the mentioned diffusion time intervals were strictly controlled so that potential differences in the coupling pattern may be attributed to physiological differences instead of experimental variations. Consequently, in tracer experiments, the injection was restricted to a single horizontal cell per retina, creating a scenario characterized by an “all or nothing” dynamic, underscoring its inherent complexity.

Subsequently, after fixation, the retina was incubated with Alexa Fluor 568 conjugated to streptavidin (Figure 2C, B). This step aimed to visualize the diffusion of neurobiotin from the injected cell to their coupled counterparts, exploiting the remarkable affinity and specificity of the avidin-biotin interaction (Laitinen *et al.*, 2006). Later, an overview of the retina was scanned (Figure 2C, C-D) to assess the experiment's success. Notably, only 14 out of 43 retinas exhibited coupled horizontal cells. In successful experiments, I could identify coupled horizontal cells (Figure 2C, D-E). These were scanned with the confocal microscope, using the LAS X navigator to capture all the coupled horizontal cells. A manual counting of the coupled horizontal cells was conducted in Fiji/Image J with the ICA LUT for improved identification

of the horizontal cell somata (Figure 2C, E). Subsequently, a convex hull was fitted around the somata of the outermost horizontal cells to determine the area covered by the coupled cells (Figure 2C, E). The coupling data obtained were subjected to a Mann-Whitney test to assess statistical significance (Figure 7). All 14 retinas containing coupled horizontal cells across the dorsonasal and ventronasal sides of the retina were reconstructed into their spherical form (Figure 2C, D) using Matlab and the R package Retistruct, as described earlier, and plotted into a heatmap (Figure 2C, G). The area covered by the coupled cells was mapped on a heatmap for further analysis.

It is worth mentioning that the execution of tracer-related experiments demands significant technical challenges. One such challenge was to keep track of retinal orientation to ensure accurate and reliable data interpretation. Furthermore, it was essential to keep the experiment under controlled conditions like temperature, pH, and light conditions. To analyze horizontal cell coupling, the light source (given by the fluorescent lamp integrated into the setup) was maintained at low levels. Since the coupling is light-dependent (see discussion), excessively bright lighting could result in retinal bleaching, rendering it unresponsive and causing gap junction closure, making coupling undetectable. Therefore, it was imperative to establish the optimal light levels at which coupling remained detectable, achieved under low-light conditions. Nevertheless, even when the experiment was conducted under appropriate light conditions, this did not guarantee a successful outcome in which the coupling could be detected. Additionally, the experiment became even more challenging due to the intricate task of identifying horizontal cell somata located deep within the inner nuclear layer, especially in low-light conditions, often hindered by the presence of the vitreous body.

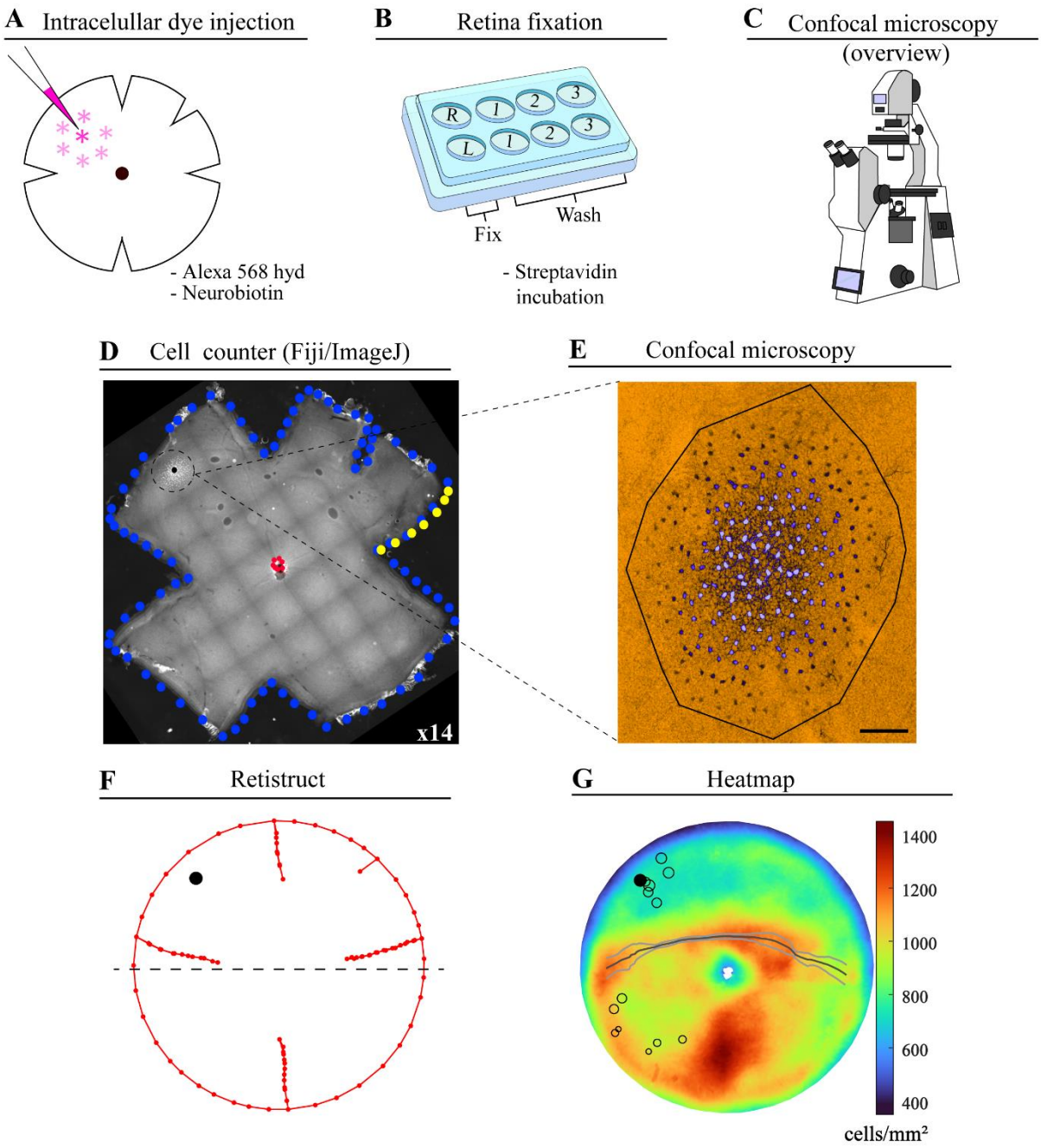


Figure 2C| Scheme illustrating the experimental protocol employed to analyze the coupling of horizontal cells. A. Intracellular dye injections with Alexa 568 hydrazide and neurobiotin within the dorsonasal and ventronasal sides of the retina. The time of neurobiotin injection and diffusion were strictly controlled. **B.** After intracellular dye injection, the retina was immediately fixed in 2% PFA and washed three times with 0.1 M PB. Subsequently, the retina was incubated overnight at 4°C with Alexa 568 coupled to streptavidin to reveal the coupled horizontal cells. **C.** An overview of the retina (shown in D) was scanned to subsequently reconstruct the retina into its original spherical shape. A total of 43 retinas were scanned. Nonetheless, only 14 were included in the analysis because the remaining ones did not exhibit coupled cells. **D.** Overview of the retina. The cell counter plugin from Fiji was used to mark the outline of the retina (blue), the optic disc (red), the temporal side (yellow), and the injected horizontal cell (black) through which the tracer was diffused. The dashed circle encloses the coupled horizontal cells located in the dorsonasal side of the retina. **E.** Confocal

microscopy scanning of the coupled horizontal cells in D, showed as a maximum intensity projection. The cells were manually counted using the ICA LUT in Fiji/ImageJ to identify horizontal cells somata better. Subsequently, a convex hull was adjusted to the outermost somata to determine the area covered by the coupled cells. Scale bar: 100 μm **F.** Illustrates the reconstructed retina shown in D. The black dot (also shown in G) represents the number of coupled horizontal cells shown in E. **G.** Illustrates a horizontal cell density map including the position of the coupled horizontal cells (open circles) within the dorsonasal and ventronasal sides of the retina from 14 tracer-injected retinas. Retina reconstructions were done by Christoph Block (Figure 2C, F-G).

8.2 Horizontal cell morphology and connectivity

Horizontal cells from a whole-mount preparation of the mouse retina were injected with Alexa 568 hydrazide to analyze their morphology. Following DAPI incubation, horizontal cell somata were identified based on their large soma and location within the INL. The mouse retina is characterized by a single type of horizontal cell, the axon-bearing horizontal cell or B-type (Peichl and González-Soriano, 1994). These cells are arranged in a mosaic pattern, and it has been described that the coverage factor of horizontal cells in the mouse retina ranges between 5 and 7 (Reese *et al*., 2005; Behrens *et al*., 2022), indicating that horizontal cell dendritic fields maintain a dendritic overlap of approximately 5-7 cells across the retina. This dendritic arrangement allows multiple horizontal cells to connect with individual cone pedicles (reviewed by Peichl *et al*., 1998).

Figure 3 illustrates the classic morphology of horizontal cells, in line with what was previously described (He *et al.*, 2000; Peichl, 2010). These cells exhibit an approximate circular profile with a uniform stellate morphology, featuring a compact dendritic tree extending in multiple directions from the soma. On average, horizontal cells possess 7 ± 1 primary dendrites (mean ± SD), which branch to establish synaptic connections with individual cone pedicles within their dendritic field (Figure 3 B-b). The distribution of these synaptic connections within the horizontal cell field does not display a uniform distribution; instead, there is a higher number of dendritic terminals innervating a cone pedicle closer to the soma than those located further away, suggesting that horizontal cells receive higher synaptic input from cones located in the proximity of their soma than those located further away (Ahnelt and Kolb, 1994a; Behrens *et al.*, 2022). This distribution is likely designed to ensure consistent anatomical coverage at the pedicles despite variations in density (Reese *et al.*, 2005).

In addition, an axon emerges from the dendritic tree of the horizontal cells, terminating in an extensive structure known as the axon terminal (Figure 3 A, C, D). This terminal system exhibits substantial branching, forming numerous evenly distributed terminals that establish exclusive connections with rod spherules (not shown) (Peichl, 2010).

In a recent publication by Behrens *et al.*, 2022, the researchers identified short segments of increased dendritic diameter on the primary dendrites of horizontal cells, termed "bulbs." Unlike the uneven distribution of horizontal cell dendritic tips throughout their dendritic tree, the bulbs were found to be uniformly distributed along the primary dendrites and across the entire dendritic field. The authors found the horizontal cell bulbs located in an OPL stratum beneath the cone axon terminal base, establishing connections with neighboring horizontal cells and ON bipolar cells. However, they did not directly contact the cone axon terminal base. The authors propose that horizontal cell bulbs represent GABAergic synapses that do not receive any input from photoreceptors. Consistent with these findings, the horizontal cells I injected also exhibited the bulbs described by the researchers (Figure 3 B-b). However, neither immunolabeling nor quantification of the bulbs was performed, as it was not the primary focus of this dissertation.

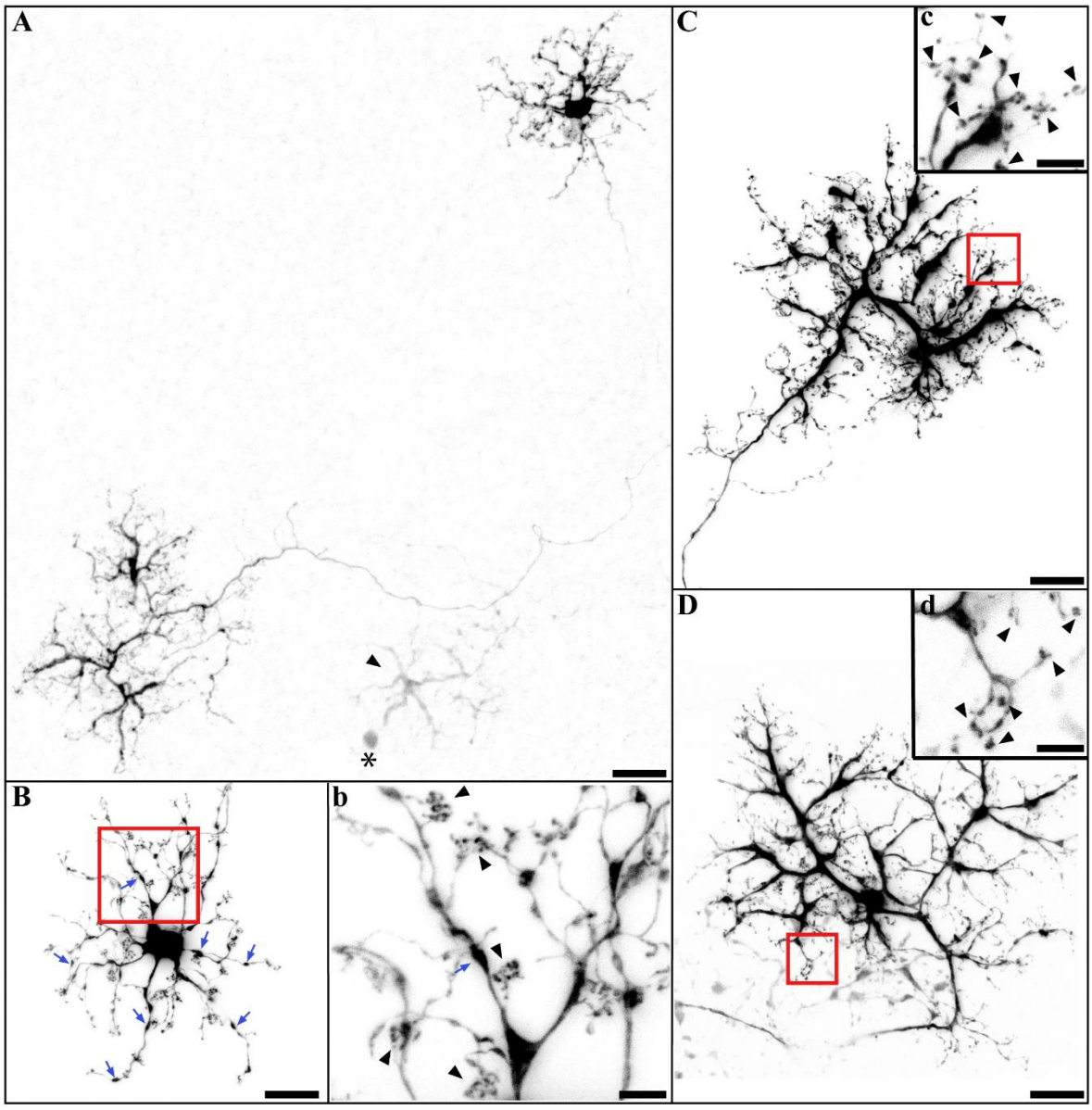


Figure 3| Horizontal cell morphology. (A) Intracellular dye injection of a horizontal cell with a fluorescent dye and a tracer molecule revealed the entire morphology of the injected cell, characterized by a compact dendritic arbor with a stellate shape and an extensive axon that gives rise to a very complex axon terminal, characteristic of the B-type horizontal cell. In some cases, axons bifurcate and give rise to two terminal systems (He *et al.*, 2000). The arrowhead points to an axon terminal from a further injected horizontal cell. The asterisk indicates auto-fluorescent dirt. **(B,b)** Intracellular dye injection with a fluorescent dye reveals the dendritic arbor of the horizontal cell. On average, 7 ± 1 (mean ± SD) primary dendrites originate from the soma and create several branches which form thin terminals that invaginate the cone pedicles. The red square corresponds to a zoom shown in (b), where the black arrowheads indicate some examples of the mentioned dendritic terminals (dendritic tips), and the blue arrows point to some of the horizontal cell bulbs. **(Cc-Dd)** Maximum intensity projection illustrates the complexity of two axonal terminals, with abundant branches that give rise to numerous terminals that contact rod spherules (not shown) (Peichl, 2010). Examples of these terminals are indicated with the arrowheads in c-d, respectively. A-D are all different cells. Scale bar 20 µm for all images except for b,c, and d, that is 5 µm.

8.3 Connexin 57 gradient across the dorsoventral axes of the mouse retina

Horizontal cells of the same type are homologously coupled by gap junctions (Weiler *et al.,* 1999; He *et al.,* 2000). The mouse retina possesses a singular type of horizontal cell (B-type) that contacts cones through its dendrites and rods through its axon terminal (Peichl and González-Soriano, 1994). In this regard, horizontal cells form two separate networks coupled by dendro-dendritic gap junctions composed of Cx57 (Hombach *et al.,* 2004; Janssen Bienhold *et al.,* 2009) and axo-axonal gap junctions composed of Cx57 and Cx50 (Dorgau *et al.,* 2015).

Consequently, since horizontal cells form extensively coupled networks, their receptive field is much larger than their dendritic field (Kaneko, 1971; Kaneko & Stuart, 1984; Skrzypek. 1984; Dacheux & Miller, 1981; Dacheux & Raviola, 1982; Vaney, 1991; Bloomfield *et al.,* 1995; Shelley *et al.,* 2006). In the mouse retina, horizontal cells are coupled through Cx57. This connexin has been reported to be exclusively expressed by horizontal cells, and it has been demonstrated that Cx57 plays a crucial role in horizontal cell connectivity since their coupling pattern is reduced by 99% in a Cx57 deficient mouse line (Hombach *et al.,* 2004). It has been described that Cx57 is expressed in both the dendrites and axon terminals of horizontal cells, suggesting that both networks are coupled through this connexin. Furthermore, it was found that the axon terminals of horizontal cells also express Cx50. However, this connexin seems strictly restricted to the axon terminals since horizontal cell dendrites do not express it. These findings suggest that horizontal cells do not form heterotypic or heteromeric channels but two independent sets of homotypic gap junctions. Consequently, both connexins likely undergo differential regulation, allowing these cells to independently adapt to varying light conditions (Dorgau *et al.,* 2015).

To date, topographical differences in the Cx57 expression have not been reported; however, unpublished data (paper in preparation) obtained by Matteo Spinelli (supervised by Dr. Christian Puller and apl. Prof. Dr. Ulrike Janssen-Bienhold, Visual Neuroscience Group, University of Oldenburg) demonstrates a Cx57 gradient across the dorsoventral axes of the mouse retina in the outer plexiform layer. Immunostainings with Cx57 in mouse vertical sections revealed higher expression of this connexin in the dorsal retina compared to the ventral retina (Figure 4 B, D). Here, it should be noted that the mouse retina expresses two types of cone opsins: M and S. These opsins create opposing and overlapping gradients across the dorsoventral axes of the retina, resulting in a majority of cones expressing both opsins (Nadal-Nicolas, 2020). In this regard, M-opsin is enriched in the dorsal retina and S-opsin in the ventral retina (Applebury *et al.,* 2000). Immunohistochemical staining for S-opsin reveals the opsin

transition zone, where the M- to S-opsin expression ratio in M-cones increases (Chang *et al.*, 2013) (Figure 4, C). Consequently, this distinctive opsin expression pattern serves as a guide for orienting the retina along the dorsoventral axis (Hughes *et al.*, 2013). Therefore, in this study, retinal regions above the S-opsin transition zone are identified as dorsal, while retinal regions below the S-opsin transition zone are identified as ventral.

The subsequent quantification of Cx57 in the dorsoventral peripheral areas of the retina, depicted in Figure 4 C, demonstrates a significant difference in the expression of Cx57 between the dorsal and ventral sides of the retina (Figure 4 D, n=3), without significant differences between the nasal and temporal sides (not shown). It is worth mentioning that the quantification of Cx57 was carried out in the dorsoventral peripheral areas since in the central retina (~25% eccentricity), Cx57 changes its expression, and this appears to be associated with the transition zone of the S-opsin gradient. Thus, to prevent any misinterpretation of the quantification data, regions at ~50% and ~75% eccentricity within the retina were selected to perform the analysis.

These findings underscore the significance of considering retinal location when investigating visually related neuronal function. The results presented here highlight a potential gradient of synaptic organization along the dorsoventral axes of the retina, raising the following question: Is the Cx57 gradient across the dorsoventral axes of the retina accompanied by changes in the dendro-dendritic coupling of the horizontal cells?

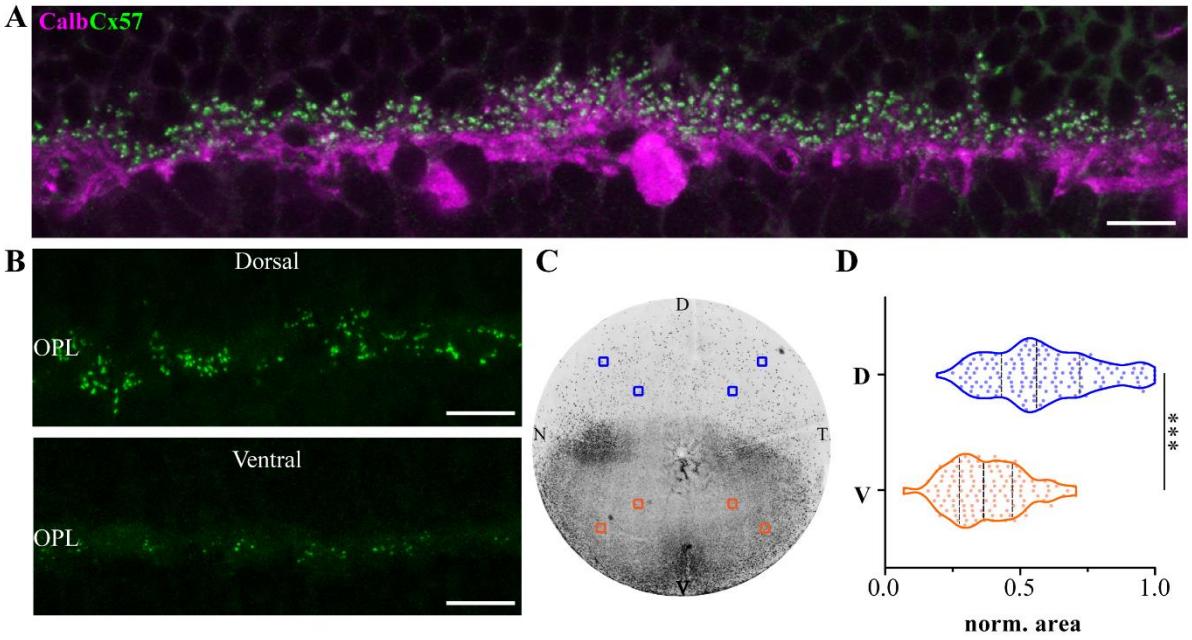


Figure 4| Cx57 expression varies across the mouse retina. (A) Vertical slide from a wild-type mouse line (C57BL6/J) immunolabeled with calbindin (magenta) and Cx57 (green), a gap junction protein. Calbindin is a calcium-binding protein commonly used to label horizontal cells

(Mitchell *et al.,* 1995). **(B)** Immunostaining labeled with Cx57 revealed a higher expression of this protein in the dorsal retina compared with the ventral retina. **(C)** Reconstruction of a whole-mounted retina and the corresponding S-opsin staining pattern. Blue and orange boxes indicate the scanned areas within the retina for subsequent quantification of Cx57 in the dorsoperipheral and ventroperipheral areas, respectively. **(D)** Violin plot showing the quantification analysis for Cx57 from the regions depicted in C, within dorsoperipheral (blue) and ventroperipheral (orange) sides of the retina (n=3 retinas). Cx57 was quantified below the cone pedicle base, which is represented by the dots (dorsal = 144, ventral = 131). An ANOVA two-tailed t-test was performed to test for statistical significance, indicating significative differences between the dorsoventral axes of the retina ($p<0.001$). Data is presented as the median and interquartile range (Q1, Q3). Analysis made by Matteo Spinelli (paper in preparation).

8.4 Horizontal cell gradient within the mouse retina

Recent advancements in imaging techniques and electrophysiological methods have enabled researchers to delve deeper into the complexities of retinal circuitry. Furthermore, understanding the morphological and functional diversity of different classes of neurons is fundamental to elucidate the neural mechanisms underlying visual processing. As previously mentioned, several studies have provided evidence regarding the morphological and functional properties of several retinal types, such as bipolar cells (Wässle *et al*., 2009; Warwick *et al*., 2018; Camerino *et al*., 2020) and ganglion cells (Hughes *et al*., 2013; Bleckert *et al*., 2014; Nadal-Nicolas *et al*., 2020; Gupta *et al*., 2023).

Several topographic differences have been demonstrated in the last decade, particularly for ganglion cells. For example, Bleckert *et al.,* 2014 demonstrated that ganglion cells of different types exhibit different non-uniform densities throughout the retina, which vary with retinal location. Furthermore, Warwick *et al.,* 2018 showed that several ganglion cell types exhibit unique density and morphology patterns. In addition, certain ganglion cell types, like the transient OFF-α retinal ganglion cells, show different light responses across different regions of the retina despite their apparently uniform distribution.

Furthermore, another study reveals that horizontal cells play a crucial role in adjusting the dynamic range of transient OFF-α retinal ganglion cells and contribute to the organization of ganglion cell receptive fields in mice (Ströh *et al.,* 2018)**.** In the same context, a recent investigation reveals that the receptive fields of retinal ganglion cells exhibit variations in their shape along the dorsoventral retinal axis, with a marked surround asymmetry at the visual horizon (Gupta *et al.,* 2023). These findings suggest that visual space is not sampled uniformly by parallel processing circuits across the mouse retina. Instead, it suggests that specific populations of retinal ganglion cells may be organized into distinct topographical arrangements

to encode specific visual features or regions. However, the relationship between these findings and the role of horizontal cells remains a subject of interest. While several studies have focused on the function of horizontal cells in modulating feedback signaling to photoreceptors and feedforward signaling to bipolar cells (Ahnelt and Kolb, 1994; Thoreson, 2008; Jackman *et al.*, 2011; Thoreson and Mangel, 2012; Vroman *et al.*, 2013; Kramer and Davenport, 2015; Behrens *et al.*, 2022; reviewed by Chapot *et al.*, 2017 and Barnes *et al.*, 2022) as well as the contribution of horizontal cells in shaping the receptive field of ganglion cells (Barlow *et al.*, 1957; Kemler *et al.*, 2014; Chaya *et al.*, 2017; Drinnenberg *et al.*, 2018; Ströh *et al.*, 2018), there is currently a notable gap in our understanding regarding topographic variations in horizontal cells, which could potentially have an impact downstream in visual processing.

Several studies have drawn attention to variations in the density of horizontal cells within the mouse retina (Wässle *et al.*, 1978; Linberg *et al.*, 1996; Reese, 2008; Camerino *et al.*, 2020). However, these studies have primarily linked density to retinal eccentricity, focusing on the central and peripheral regions of the retina while neglecting the potential existence of heterogeneous variations in horizontal cell density across distinct retinal regions.

Taking this into account, an experiment conducted by Matteo Spinelli, Dr. Christian Puller, Lucia Lindenthal, and Christoph Block aimed to immunolabel and quantify horizontal cells across all retinal axes in whole-mount preparations to potentially unveil topographic variations of these cells. Thus, five retinas were immunolabeled with calbindin, a calcium-binding protein used as a marker for horizontal cells (Mitchell *et al.*, 1995). Individual horizontal cells somata were manually counted in all retinas (Figure 5A). Subsequently, the retinas were reconstructed into their original spherical shape (Figure 5B). The average horizontal cell density across different retinas was calculated in a fixed, regular grid using Matlab. The results were computed and plotted into a heatmap as mean ± standard deviation, including a representation of the S-opsin transition zone (Figure 5C). The outcome of this experiment provides visual evidence of a non-uniform horizontal cell gradient across the dorsoventral axes of the mouse retina. Specifically, horizontal cells in the dorsal retina (above the S-opsin transition zone) exhibit lower density than those in the ventral retina (below the S-opsin transition zone). However, increased densities were found in a region aligning with strong S-opsin expression (Figure 4 C, Figure 5 C). Moreover, a horizontal cell hotspot was found in the ventral retina, potentially overlapping with the S-true cone gradient described by Nadal-Nicolas *et al.*, 2020. A similar hotspot was described by Camerino *et al.*, 2020 for bipolar cell type 3b (see discussion).

The findings provided here suggest compelling evidence of an undiscovered retinal specialization, reinforcing the notion that the visual system may adapt to varying visual demands based on specific visual tasks (see discussion).

Consequently, variations in the expression of Cx57, together with the horizontal cell gradient across the dorsoventral axes of the mouse retina, raise the following question: Does the electrical coupling of horizontal cells also exhibit variations within the different axes of the retina?

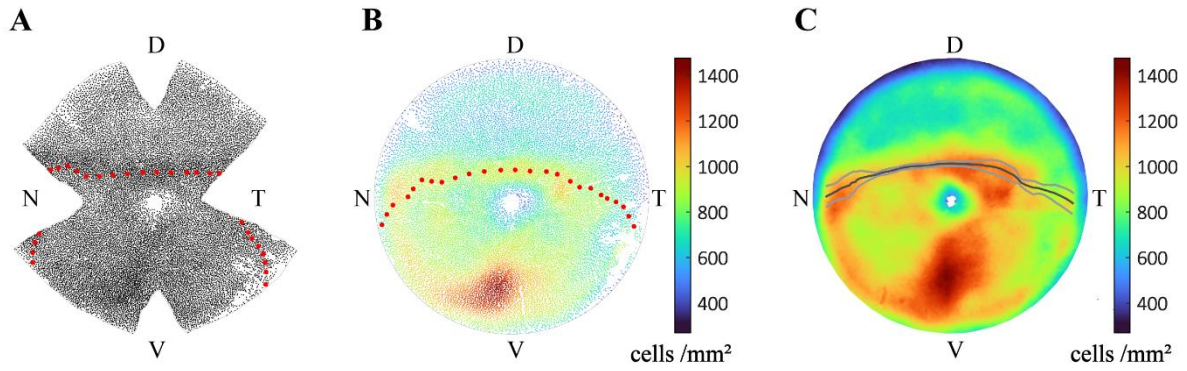


Figure 5| Horizontal cell density map. (A) Whole-mount dissection of a mouse's left retina. Every dot represents a horizontal cell soma labeled with calbindin. Red dots represent the S-opsin transition zone. The retinal axes are depicted as D (dorsal), V (ventral), T (temporal), and N (nasal). **(B)** Azimuthal equal-distance projection of a reconstructed retinal sphere. Local density is represented by colors where dark (bluish) colors represent low-density areas, and bright (reddish) colors represent high-density areas (see color scale). **(C)** Average density distribution of 5 retinas showing a horizontal cell gradient across the dorsoventral axes of the retina. The S-opsin transition zone is illustrated as mean (black line) ± standard deviation (grey lines). Interestingly, horizontal cells in the dorsal retina (above the S-opsin transition zone) are less dense than those in the ventral retina (below the S-opsin transition zone). However, higher densities were found in a region aligning with strong S-opsin expression (Figure 4 C). In addition, there is a remarkable horizontal cell hotspot in the ventral retina. The horizontal cell density map from the figures below illustrates the same orientation represented in this figure. This work was done by Matteo Spinelli, Dr. Christian Puller, Lucia Lindenthal, and Christoph Block (paper in preparation).

8.5 Horizontal cell coupling varied along the dorsoventral axis of the retina

To delve deeper into potential variations in horizontal cell electrical coupling, experiments involving intracellular dye/tracer injections with Alexa 568 hydrazide and neurobiotin, respectively, were performed on the peripheral nasal side of the retina (Figure 6, A), as the horizontal cell gradient showed more prominent variations in the nasal side compared to the temporal side (Figure 5, C). Notably, 14 out of 43 tracer-injected retinas showed coupled horizontal cells across the dorsoventral sides of the retina (see discussion).

To determine the area covered by the coupled horizontal cells, a convex hull was adjusted around the outermost horizontal cells somata, enclosing the area covered by the coupled cells. The results of these experiments revealed substantial differences in the horizontal cell coupling between the dorsoventral axes of the retina. Specifically, the dorsonasal side exhibited a higher number of coupled cells (233 ± 45), covering a larger area (0.24 ± 0.04 mm^2). In contrast, the ventral side exhibited a smaller number of coupled cells (130 ± 46), and also the area covered by these cells was considerably smaller (0.11 ± 0,05 mm^2) (Figure 6 B, C). A Mann-Whitney test was performed to determine the statistical significance of these findings. This test demonstrated a significant difference along the dorsoventral axes of the retina in both parameters, the number of coupled cells ($p=0.0023$, Figure 6 D) and the area covered by them ($p<0.001$, Figure 6 E). Taken together, these results align cohesively with the differential Cx57 expression across the dorsoventral axes of the retina. Thus, the dissimilar coupling observed between dorsonasal and ventronasal regions suggests that the differences between the number of coupled cells and the area covered by them are not solely attributable to cell density but reflect a different physiological regulation characterized by increased electrical coupling of the horizontal cells in the dorsal side, covering larger fields and in consequence, encompassing broader areas. Conversely, a decreased expression of Cx57 of the horizontal cells on the ventral side translates into less coupled cells covering smaller fields. Such variations might potentially have an impact on downstream neurons, supporting the idea that horizontal cells shape the receptive fields of bipolar cells and ganglion cells differently across the dorsoventral axes of the retina.

Alternatively, another perspective that might explain the striking differences in the coupling pattern of horizontal cells between the dorsoventral axes pertains to their size. Specifically, horizontal cells with bigger dendritic trees would encompass larger areas compared to their smaller counterparts. Thus, the following question is addressed: Could the observed disparity in the horizontal cell coupling potentially be attributed to variations in their dendritic field size?

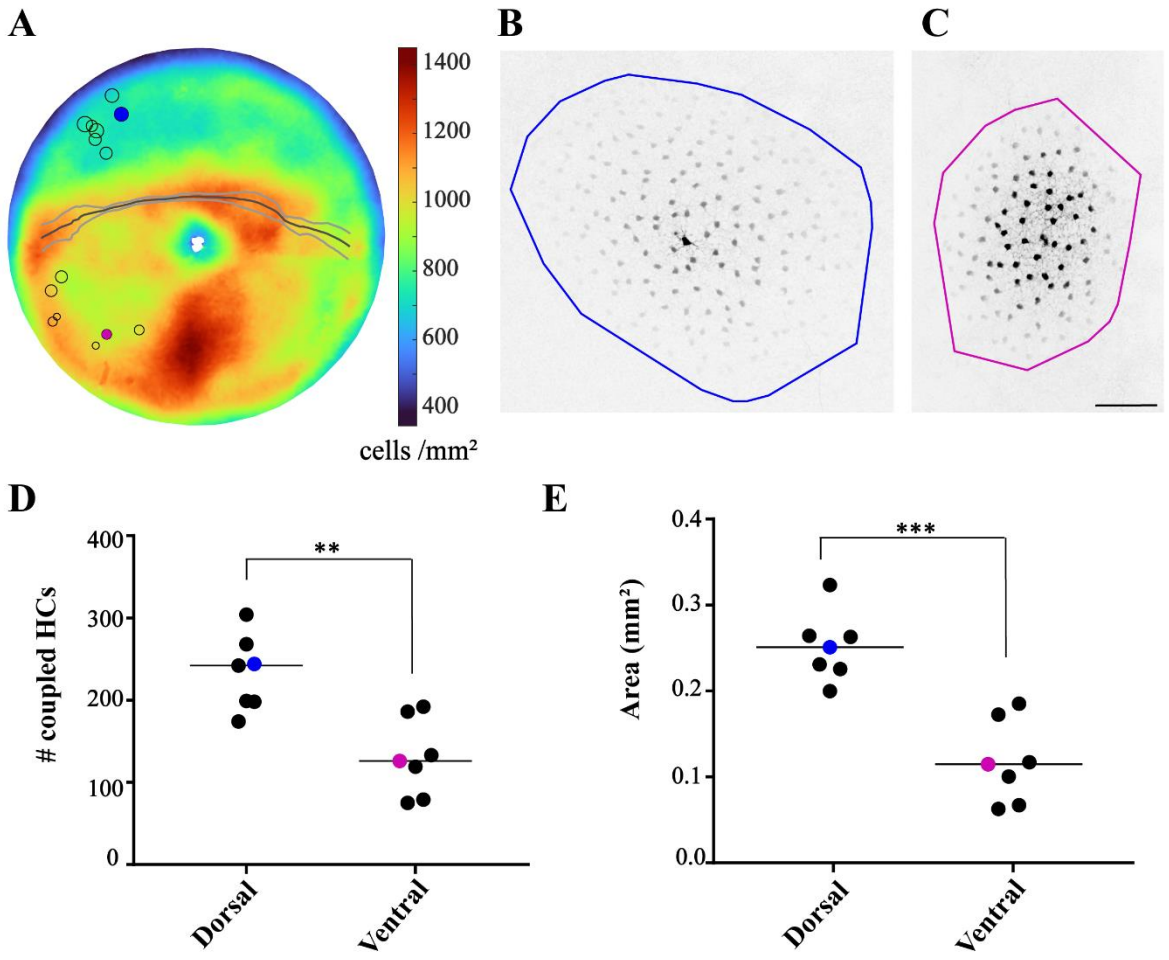


Figure 6| Horizontal cells show different coupling between the dorsoventral axes of the mouse retina. (A) Horizontal cell density map including a representation of the number of coupled horizontal cells (black circles) and their location within the retina. Blue and magenta-filled circles represent the horizontal cell coupling shown in B and C, respectively. **(B-C)** Intracellular neurobiotin injection of horizontal cells from dorsal (B) and ventral (C) retina from Black 6 (wild type) mice. The blue (B) and magenta (C) outlines represent a convex hull adjusted to the outermost coupled horizontal cells, enclosing the area covered by them. It is worth mentioning that the tracer becomes more diffuse with greater distance from the injected cell. Scale bar 100 µm. (B and C not scaled with respect to A). **(D-E)** Scatter plot showing the number of coupled horizontal cells (D) and the area they cover (E) between the dorsal and ventral retinas. Consequently, blue and magenta dots represent the data from the coupled cells shown in B and C, respectively. A Mann-Whitney test was performed in both cases to test for statistical significance ($p>0.05$). Data was obtained from 14 retinas (11 mice), and the results are presented using the median as a means of comparison. ($p<0.001$ and $p=0.0023$ for D and E, respectively).

8.6 Horizontal cells located in high-density areas possess smaller dendritic areas

Therefore, to test whether these cells showed differences in their dendritic field size, intracellular dye injections with Alexa hydrazide 568 were performed by targeting different horizontal cells located in low- and high-density areas across the entire retina. In total, 123 horizontal cells in 37 retinas were injected. Among these, 13 retinas, including 26 injected horizontal cells, were discarded because of damaged tissue, where the outline of the retina did not look reliable. In consequence, it could potentially lead to mistakes when reconstructing the retina into its spherical shape. In addition, four retinas containing eight injected horizontal cells were discarded due to the use of an enzyme solution to attempt to remove the vitreous body. It was unlikely that this solution could affect the morphology of the horizontal cells; however, since there was some uncertainty about it, the four retinas mentioned were left aside.

Of the remaining 20 retinas containing 89 injected horizontal cells, 20 cells were excluded due to issues like strong co-injections or dye leakage, which prevented morphological reconstruction. Consequently, 69 horizontal cells from 18 retinas distributed across all retinal areas were used to compare the dendritic fields. From this pool, 32 cells were randomly reconstructed to further perform a Sholl analysis (see Figure 8-9). Among these, nine reconstructed cells were discarded by a second observer due to co-injections or the presence of blurry dendrites resulting from confocal scanning, leaving 23 cells for further evaluation. The aforementioned exclusion criteria were implemented to enhance the reliability and accuracy of the results presented below.

To discern potential differences between horizontal cells, the first step was to determine the individual dendritic area for all injected cells. To achieve this, the soma and outermost dendrites were demarcated on a maximum intensity projection image of each cell (stack size ~ 20 µm) using the cell counter plug-in in Fiji/Image J. Subsequently, a convex hull was fitted to each cell (properly scaled) using Matlab to determine their respective dendritic area. As mentioned before, 69 injected horizontal cells were analyzed and plotted onto the horizontal cell density map after retinal reconstruction (Figure 7 A, by Christoph Block). The results unveiled noticeable differences in dendritic size across the dorsoventral axes of the retina. These findings suggest that horizontal cells with extensive dendritic arbors were primarily located in the dorsal retina and toward peripheral regions in low-density areas. Conversely,

horizontal cells with more compact dendritic arbors were predominantly located on the ventral side, often in areas characterized by high densities.

To gain deeper insights into the relationship between the dendritic area of horizontal cells and their density, a scatter plot was generated based on these two variables (Figure 7 B). The outcome suggested a correlation between the dendritic area and the horizontal cell density, indicating that cells located in high-density areas tend to be smaller compared to those located in low-density areas. Consequently, the predicted coverage factor in this study was determined by multiplying the spatial density of the cells (cells/mm^2) by the dendritic field area of each individual cell (mm^2/cell) (Reese, 2005; Masland, 2012). This resulted in a coverage factor of 5, suggesting that horizontal cell dendritic fields adjust their size to maintain a dendritic overlap of around five cells throughout the retina (Figure 7). This result slightly differs from what was described previously by Reese *et al.*, 2005, where the authors reported a coverage factor of 6 and 7 were found in the albino and wild-type mice, respectively.

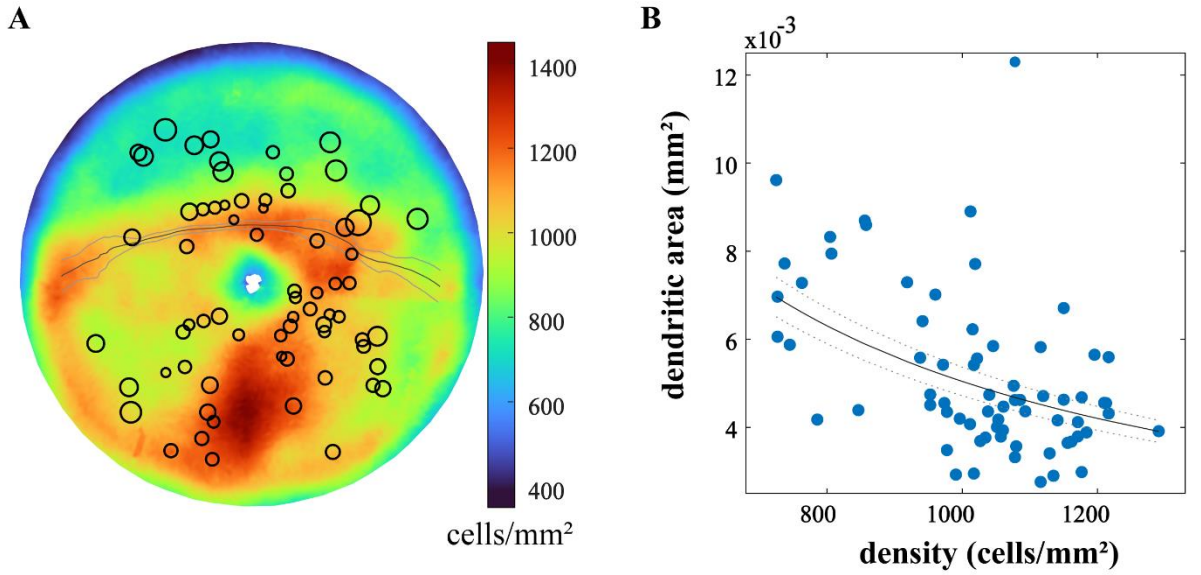
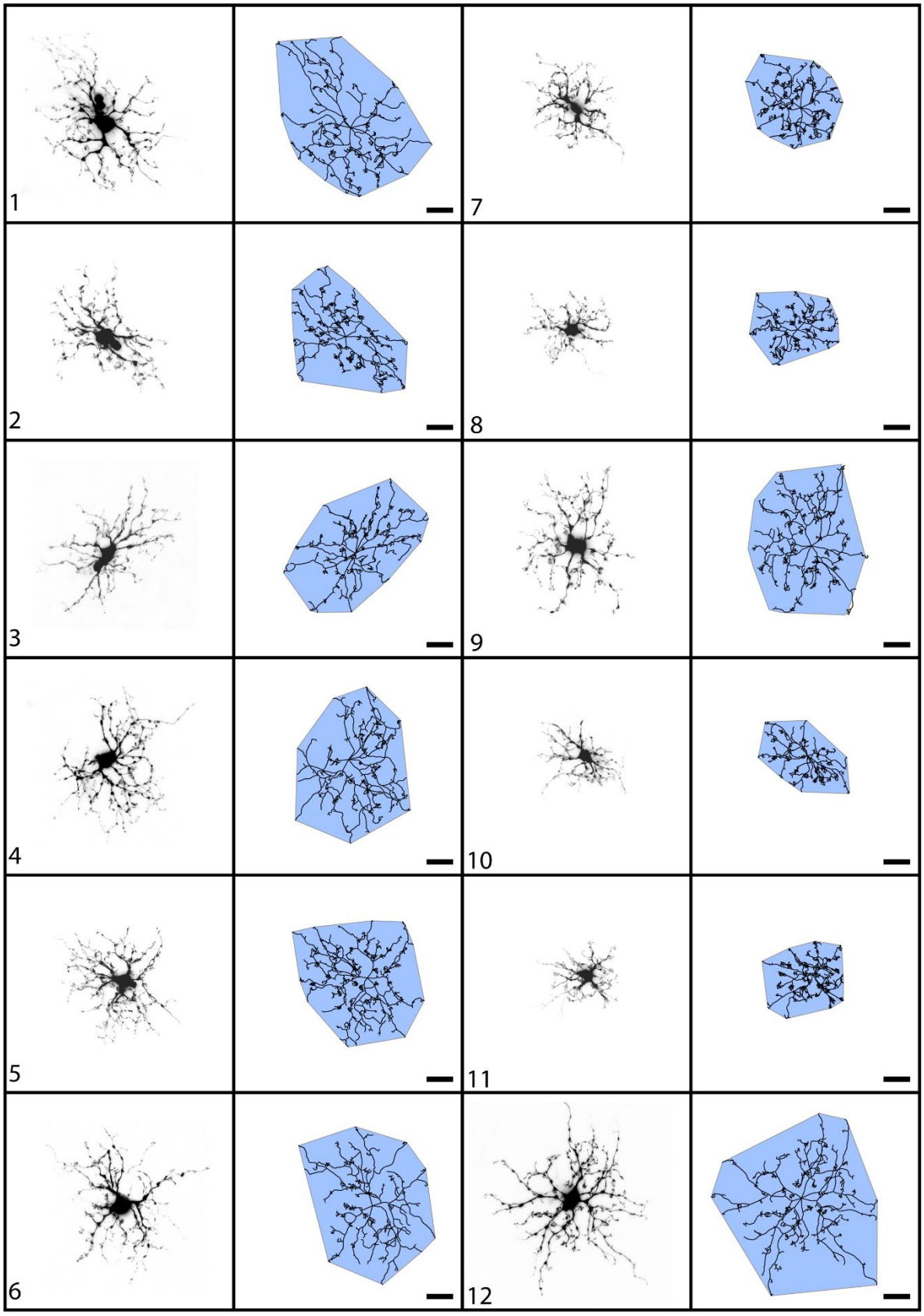


Figure 7| Correlation between horizontal cell density and their dendritic area. (A) Horizontal cell density map including a representation (black circles) of the dendritic area of each of the injected cells and their location within the retina (n=69). Notably, horizontal cells located in peripheral and dorsal regions exhibit larger dendritic areas compared to those located in the ventral region. **(B)** Correlation diagram for the relationship between dendritic area and density of the injected horizontal cells. The predicted coverage factor was calculated by multiplying the spatial density of the cells (cells/mm^2) by the dendritic field area of each individual cell (mm^2/cell) (Masland, 2012), resulting in a coverage factor of 5. The black line represents the 95% confidence interval (CI), while the dotted blue lines represent the lower and upper limits, respectively. (95%CI [4.72,5.37]). Data plotted by Christoph Block.

The morphologies of all the reconstructed horizontal cells are shown in Figure 8. Convex hulls were adjusted to the outermost dendrites to determine the dendritic area. Differences in the dendritic tree size are easily recognizable between horizontal cells from dorsal vs. ventral regions, where, in general, cells located in low-density areas possess a bigger dendritic tree than cells located in high-density areas. For instance, horizontal cells numbered 15, 16, and 23 are located in high-density areas and consequently smaller than those in the ventral periphery, such as horizontal cells numbered 17 and 18. Consequently, the area covered by horizontal cells numbered 15, 16, and 23 is smaller compared to horizontal cells numbered 17 and 18 (Figure 8). This observation roughly follows the normal size distribution shown in Figure 7. On the other hand, horizontal cell reconstructions reveal the intricacy of their morphology, notably characterized by a complex dendritic tree with an exhaustive branching pattern and small dendritic tips that intricately invaginate the cone terminals. Consequently, the reconstruction of these cells presents considerable challenges in contrast with other neuronal classes, such as M1- and M2 -type intrinsically photosensitive ganglion cells (ipRGCs) (Duda *et al.,* 2023), whose dendritic pattern exhibits minimal branching.

The observation about differences in the dendritic size of horizontal cells distributed across the dorsoventral axes of the retina raises an intriguing question: Are these cells fundamentally distinct entities or merely smaller versions of their larger counterparts?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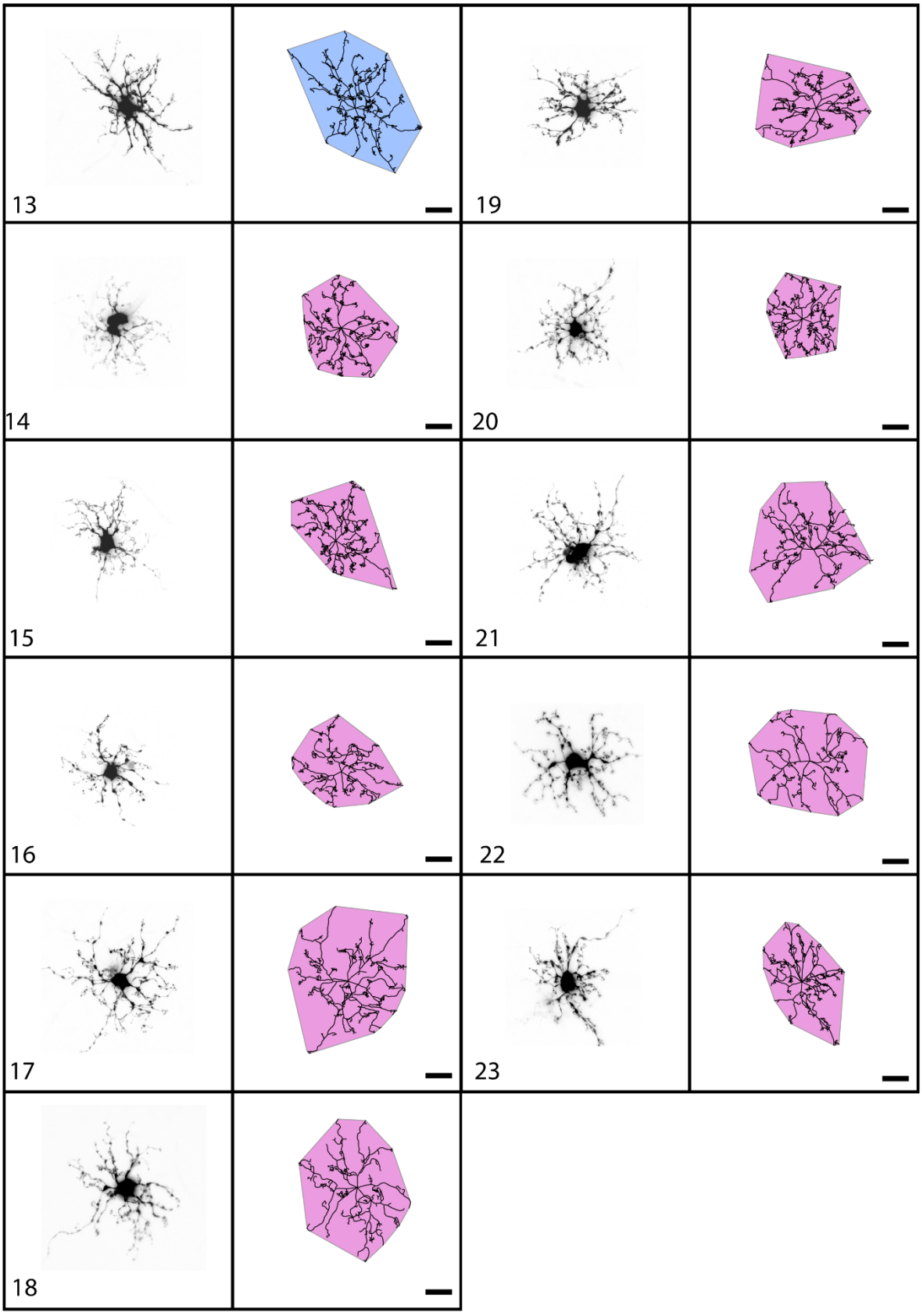
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Figure 8 | Horizontal cell dendritic field reconstructions. The left panel shows the morphology of 23 intracellularly injected horizontal cells from different areas of the retina (see Figure 9 A). Differences in the size are easily recognizable. The right panel shows the reconstruction of the respective horizontal cell on the left side. A convex hull was fitted to the reconstructions to determine the horizontal cells dendritic area. The numbers indicate the location of the cell on the horizontal cell density map shown in Figure 9-A. Blue refers to horizontal cells located on the dorsal side (above the S-opsin transition zone), and magenta indicates horizontal cells located on the ventral side (below the S-opsin transition zone). The axon was not included in the convex hull since it leads to an overestimation of the dendritic field. Scale bar 20 µm.

8.7 Horizontal cells do not differ in their branching pattern or number of termination points, regardless of their size.

To gain a better understanding of the morphology of the horizontal cells, particularly regarding their branching pattern and number of termination points (dendritic tips), a Sholl analysis was performed (Sholl,1953). The aim was to compare the arborization pattern of all 23 reconstructed horizontal cells shown in Figure 8. These cells were plotted onto the horizontal cell heatmap, and their retinal location is illustrated in Figure 9-A.

The raw data from the Sholl analysis is presented in Figure 9-C, revealing subtle variations in the branching pattern and the number of dendritic tips among cells. However, upon normalization to the same convex hull area (Figure 9-D), the outcome suggests no apparent asymmetries in the number of dendritic intersections or termination points. Consequently, these findings suggest a remarkable similarity in dendritic fields among individual horizontal cells. Regardless of their size, there is no discernible variation in the number of intersections or termination points among the cells, implying that horizontal cells are essentially identical to one another.

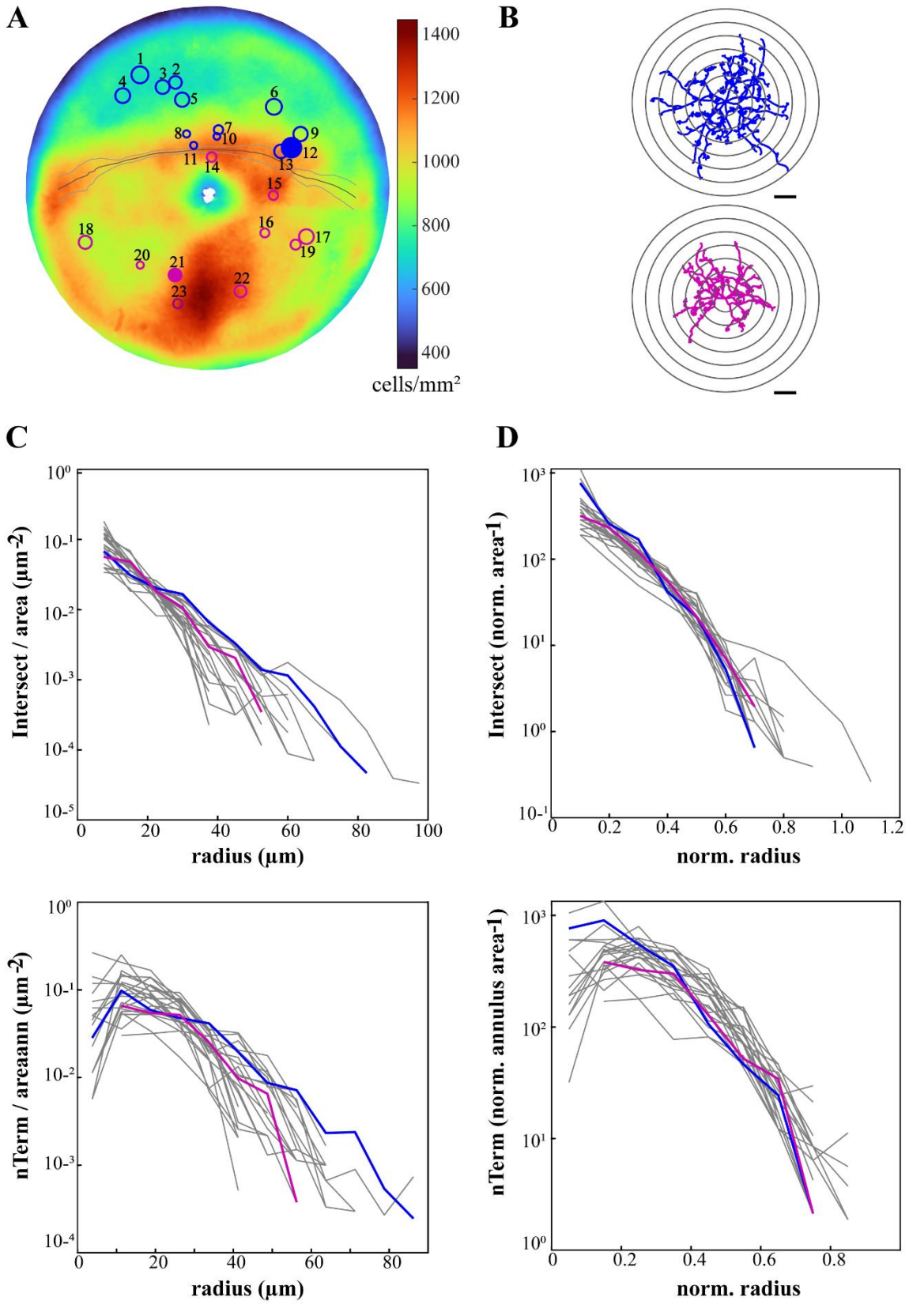
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Figure 9 |Horizontal cells do not differ in their branching pattern or number of termination points regardless of their size. (A) A horizontal cell heatmap, including the retinal location of all 23 reconstructed cells, is shown in Figure 8. Blue (dorsal) and magenta (ventral) circles illustrate the area of each reconstructed cell to simplify their comparison. Filled circles represent the horizontal cells depicted in B from the dorsal and ventral sides, respectively. **(B)** Example of a 2D Sholl analysis for the cells filled in A. Concentric circles are overlaid on the horizontal cell reconstructions, providing an insight into the complexity of their dendritic arbor. Both cells are also depicted in C-D with their respective colors for comparative purposes. Scale bar 20 µm. **(C)** Raw data for the number of intersections and termination points. The number of intersections of dendritic structures (branching pattern) with concentric circles was divided by the area of the respective circle. Similarly, the number of termination points was divided by the annulus area. To eliminate the influence of area, each cell was normalized to an equal convex hull area. The normalized data is shown in **(D).** These results suggest that regardless of their size and location within the retina, horizontal cells do not change their branching pattern or number of termination points.

9. Discussion

The results described above are currently summarized as a manuscript for publication. Nevertheless, the constrained length of scientific papers limits our ability to provide an in-depth discussion of certain aspects addressed in this study. Therefore, this section is dedicated to offering a more thorough examination of the results previously presented.

9.1 Methodological considerations

9.1.1 Retinal cuts

As mentioned before, one of the key steps in this study involved keeping track of retinal orientation. As described in Figure 2A and the “Methods” section, two horizontal cuts were meticulously positioned along the choroid fissure, followed by two vertical cuts and one last reference cut at the dorsotemporal side to be able to identify the retinal orientation. While the highest possible precision was applied when placing these cuts, natural variations in retinal tissue must be considered. Thus, it is possible to find slight variations in the (horizontal) position of the choroid fissure between mice. In addition, human error in executing the cuts must be considered, as achieving exact uniformity in cut placement across different retinas can be challenging.

These two introduced errors are important to consider when aligning and reconstructing the retina into its spherical form using Retistruct. These errors could potentially cause slight variations in the position of the horizontal cells when traced back into the heatmap. Nevertheless, it is noteworthy that these variations did not significantly impact the results previously presented, as the Sholl analysis (Figure 9) did not reveal striking differences in the branching pattern or termination points between horizontal cells located in different retinal areas.

In addition, a certain degree of shrinkage after tissue fixation with PFA may occur (Werner *et al.*, 2000; Shepherd *et al.*, 2009; Wehrl *et al.*, 2015); however, this does not significantly impact the outcomes presented in this study as shrinkage does not have an impact on the horizontal cell distribution, arborization, or coupling pattern and the degree of shrinkage is likely similar for all specimens.

9.1.2 Intracellular dye/tracer injection

In general, when conducting experiments such as electrophysiology or intracellular injections, it is essential to design appropriate electrodes for different targets (e.g., neurons, embryonic cells, fertilized eggs). Horizontal cell injections can be particularly challenging, as the cells are located deep within the INL. Thus, intracellular dye injections are hard to achieve in retinal whole-mount preparations (Meyer *et al.*, 2018). In this regard, it becomes imperative to design electrodes with exceptionally fine tips, adequate length, and strength to penetrate the tissue deep down to reach the horizontal cell somata.

In this study, a horizontal cell gradient across the dorsoventral axes of the retina was found, with a notable hotspot at the ventral side. Attempts to inject cells in the hotspot constantly failed, likely due to the high cellular density present in that area. In addition, efforts to penetrate the tissue by slightly forcing the electrode resulted in tissue disruption. These effects were only observed in the ventral retina in high-density areas.

To investigate this phenomenon further, a couple of control experiments were conducted. These experiments involved initial injections of neurobiotin into the ventral horizontal cell hotspot, followed by injections into the dorsal retina and a subsequent return to the hotspot. Interestingly, coupled horizontal cells were only observed on the dorsal side, suggesting that the retina remained responsive. However, it remains unclear whether the horizontal cells located in the hotspot were genuinely uncoupled or if the experiments failed due to tissue

damage, potentially leading to the closure of gap junctions and subsequently preventing the diffusion of neurobiotin to neighboring horizontal cells.

Overall, while the electrodes employed in this study proved adequate to analyze the morphology and coupling pattern of horizontal cells in the retina, it may be that a redesigned electrode, perhaps featuring a finer tip, could enhance the capability to inject horizontal cells located in the hotspot.

9.1.3 Tracer coupling

As one of the aims of this study was to analyze the coupling pattern of horizontal cells within the dorsoventral axes of the retina, intracellular tracer injections using neurobiotin were performed. Neurobiotin is a small molecule able to pass through gap junctions; thus, after immunostaining with streptavidin (coupled to a fluorescent dye), it is possible to see the coupled counterparts of the injected cell. Cut loading experiments where the retina is cut with a blade embedded in a molecular tracer demonstrate that the extent of neurobiotin diffusion through gap junctions can vary, depending on the duration of diffusion permitted by the researcher (Choi *et al.,* 2012; Myles and McFadden, 2022). Therefore, in this study, strict control over tracer injection and diffusion times was imperative to ensure the reliability of the results. Consequently, it is unlikely that the observed variations in the horizontal cell coupling pattern between the dorsoventral axes of the retina were a consequence of experimental variations. Instead, these differences could be attributed to physiological variations resulting from 1) distinct patterns of Cx57 expression across the dorsoventral axes, 2) different regulation of gap junctions across the dorsoventral axes of the retina, influencing their permeability, conductance, and gating properties or 3) a mix of both (see below).

Typically, while immunoreactivity assays serve as valuable tools for detecting protein expression, they may not always accurately reflect expression levels. Therefore, it is essential to interpret the results cautiously and consider potential factors that could affect the correlation between immunoreactivity and actual expression levels (Barrantes-Freer *et al.,* 2015). Figure 4 illustrates a gradient in Cx57 across the dorsoventral axes of the retina. However, this gradient may not necessarily suggest a different coupling pattern between horizontal cells, as the controlled opening of hemichannels and gating properties of complete gap junctions can be regulated by post-translational modifications of connexins. Post-translational changes can

affect not only channel gating, but also connexin trafficking and assembly into hemichannels (Pogoda *et al.*,2016), and these changes could vary across different retinal axes.

In this study, tracer-related experiments revealed variations in the coupling pattern of horizontal cells along the dorsonasal axes of the retina (Figure 6), demonstrating a correlation between the Cx57 gradient and the extent of coupling. Interestingly, fewer coupled horizontal cells were found in the ventral retina compared to the dorsal retina, which exhibited more coupled cells. One possible explanation could be attributed to a reduced permeability of gap junctions composed of Cx57 in the ventral retina relative to the dorsal retina. Gap junctional permeability depends on factors such as size, charge, and reactivity, which are critical for tracer flux (Mills and Massey, 2000). Therefore, a different permeability of gap junctions in the ventral retina may retain the tracer within the injected cell, reducing horizontal cell coupling.

On the other hand, it is worth noting that the tracer-related experiments displayed an "all or nothing" outcome, where either coupling was observed or not, leaving no middle ground. Notably, 14 out of 43 retinas showed coupled horizontal cells. In this regard, it is essential to emphasize that any retinas excluded from the analysis were discarded due to a complete absence of coupling. None of the retinas were discarded because of weak coupling, and there was no bias in selecting data to favor optimal outcomes. The tracer-related experiments had two potential outcomes: either horizontal cells were coupled or not.

The absence of horizontal cell coupling observed in 29 retinas may be attributed to several factors. Initially, experiments were conducted under high-light intensities to enhance the visibility of horizontal cell somata. However, given that horizontal cell coupling may be influenced by variations in light levels (Xin and Bloomfield, 1999; reviewed by Thoreson and Mangel, 2012), the use of high-light intensities might have led to retinal bleaching (Pepperberg, 2003), potentially affecting the opening and closure of gap junctions, resulting in the absence of coupling. Therefore, keeping the retina under low-light conditions was essential, even when injecting it under visual control with the fluorescent lamp on. Another potential reason why no coupling was observed could be attributed to the dissection quality. Considering the delicate nature of the retina, unintentional damage (for example, while removing the vitreous body) could lead to sporadic death or render the retina unresponsive. Thus, a fundamental requirement was to execute precise, tidy, and cautious dissections of the retina.

It is essential to highlight that the duration of light exposure for the tracer-injected retinas varied across experiments. This discrepancy arose simply because, in certain instances, it was easier to identify the horizontal cell somata due to more extensive removal of the vitreous body, resulting in enhanced DAPI staining and clearer visualization of the cells. However, despite these variations in light exposure duration, no correlation was observed between the duration of light exposure and the extent of horizontal cell coupling (data not shown).

9.1.4 Horizontal cell reconstructions

To analyze the morphology of the horizontal cells, their skeletons were reconstructed using the Amira software. The primary challenge arises from the intricate task of tracing independent dendritic tips, especially those located deep within the confocal image stack and those located at the central region of the cell. There is a potential to underestimate certain horizontal cells dendritic tips invaginating the cone pedicles. This underestimation can be attributed to a saturated soma filled with an intracellular dye, which obscures fine dendritic structures located slightly above the soma. It is essential to mention that this underestimation applies to all the reconstructed horizontal cells and did not affect the previously presented results.

Furthermore, the reconstruction of these cells becomes even more demanding due to the overlapping nature of dendrites within the cell, complications arising from co-injections that make interpretation difficult, blood vessels causing a blurred view, and occasional wobbling or drift during confocal scanning. As a result, 9 out of 32 initially reconstructed cells were excluded from further analysis by a second observer due to the abovementioned issues. Consequently, the remaining 23 reconstructed cells were deemed more reliable for subsequent Sholl analyses.

9.2 Functional meaning of the findings in this study

9.2.1 Gradients across the mouse retina

In the past, there was a prevailing notion that the mouse retina constituted a homogeneous tissue (Dräger and Olsen, 1981; Jeon *et al*., 1998). Consequently, several studies overlooked the existence of non-uniformities across the mouse retina. As a result, the precise retinal location from where data were collected often went unreported. Recent discoveries, however, provide evidence that the mouse retina displays topographic variations in a variety of forms and patterns. For instance, ON α-like retinal ganglion cells exhibit a nasotemporal gradient in

cell density, size, and receptive fields, facilitating enhanced visual sampling within frontal visual fields. (Bleckert, *et al.,* 2014). Additionally, a recent investigation mapped the distribution of all identified OFF- bipolar cell populations (1a, 1b, 2, 3a, 3b, 4). The findings revealed significant variation in the distribution of these cells along both the dorsoventral and centroperipheral axes of the retina, potentially correlating with specialized functions associated with each cell type (Camerino *et al.,* 2020). Moreover, electrophysiological recordings conducted on transient OFF- α retinal ganglion cells reveal that the responses of these cells become gradually more sustained across the dorsoventral axis of the retina. These findings suggest that the networks underlying retinal ganglion cell responses may adapt across retinal locations to facilitate optimal sampling of visual information. (Warwick *et al.,* 2018). Furthermore, recent research demonstrates that the receptive fields of retinal ganglion cells change their shape across the dorsoventral retinal axis, with a marked surround asymmetry at the visual horizon. This observation suggests that these changes might offer a simple mechanism for adapting the visual system to the constraints of natural scenes, and it could potentially influence how information is encoded by these cells (Gupta *et al.,* 2023). Conversely, a separate study identified variations in the distribution of true-S cones (expressing S-opsin only) along the dorsoventral axis of the retina. Specifically, true-S cones were found to be concentrated in the ventral retina together with S-cone bipolar cells (connecting to S-cones), whose wiring patterns correlated with the distribution of true-S cones. This unique connectivity pattern between true-S cones and S-cone bipolar cells serves as the foundation for mouse color vision, potentially representing an evolutionary adaptation to enhance color discrimination in the upper visual field, suitable for mice's habitat and behavior. (Nicolas-Nadal *et al.,* 2018). In summary, the findings mentioned above exemplify how different cell types perform unique functions across different retinal axes, allowing them to meet the diverse demands of the visual field. These results emphasize the importance of considering differences in density, morphology, and function when studying specific cell types or neuronal activity within the retina.

On the other hand, topographical variations of different retinal types found in the mouse retina challenge the conventional belief that the mouse retina does not possess retinal specializations. Instead, there is substantial evidence about the potential existence of a specialization resembling an area centralis in the ventral retina, as suggested by Heukamp *et al.,* 2020. The researchers pointed to several pieces of evidence that support this notion. First, it has been demonstrated that the M-S opsin gradient across the dorsoventral axes of the retina creates

color vision in the ventral retina but not in the dorsal side (Breuninger *et al.*, 2011; Denman *et al.*, 2018; Szatko *et al.*, 2019). This opsin co-expression may also provide functional benefits, such as enhancing contrast detection against different spectral backgrounds (Yin *et al.*, 2006). Second, the ventral retina contains a higher density of ganglion cells, where several ganglion cell types show higher densities in that area. For example, the W3 and the transient OFF-α retinal ganglion cell types are considered potential candidates for encoding approaching dark objects (Münch *et al.*, 2009; Zhang *et al.*, 2012). In consequence, the spatial distribution of these cells may help the animal recognize predators as birds coming from the sky. Third, specific wild-house mice (*Mus musculus*) display an organized pattern of high-density cone clusters restricted to the ventral retina (Warwick *et al.*, 2018), indicating that the mouse retina might possess distinct and specialized functions. Finally, while objects in the lower visual field may be processed by other senses like olfaction or whisking, objects in the upper visual field heavily rely on vision for detection (De Franceschi *et al.*, 2016).

Following the abovementioned findings, the results outlined in this study support the initial idea proposed by Heukamp *et al.*, 2020. In collaboration with Matteo Spinelli, this dissertation provides evidence of a horizontal cell gradient that apparently overlaps with the S-opsin gradient, featuring a notable hotspot in the ventral retina. Interestingly, the horizontal cell hotspot appears to overlap with the true-S cones gradient described by Nadal-Nicolas *et al.*, 2020. In addition, Camerino *et al.*, 2020 reported a non-uniform distribution of all OFF-bipolar cell types. Remarkably, they found high densities in the ventral retina for the OFF-bipolar cell 3b, accompanied by a notable hotspot, which could potentially overlap with the horizontal cell hotspot identified in this study. The mentioned bipolar cell type could potentially provide an alternative fast rod pathway (Mataruga *et al.*, 2007).

9.2.2 Functional implications of differential horizontal cell coupling across the mouse retina.

Horizontal cells are laterally interneurons that modulate the output of photoreceptors. These cells play diverse roles in early visual processing, such as color opponency, contrast enhancement, and the organization of center-surround receptive fields in cones, bipolar cells, and ganglion cells. In the past, it was commonly thought that horizontal cells were primarily involved in global signal processing (Thoreson & Mangel, 2012). However, recent findings suggest that horizontal cell feedback may also operate on a smaller spatial scale between a single horizontal cell dendritic tip and a cone axon terminal (Jackman *et al*., 2011; Vroman *et al.,* 2014). Therefore, horizontal cells may contribute to both local and global visual signal processing.

The fundamental mechanism underlying global signaling processing lies in lateral inhibition: the light-evoked activity of one photoreceptor is inhibited by the responses of surrounding photoreceptors, resulting in a center-surround organization to the receptive field. In this setup, light falling on the center excites the cell, and light falling on surrounding regions inhibits the cell (Hartline *et al.,* 19563; Hartline & Ratliff, 1957). This inhibitory mechanism functions on a global scale and adjusts cone output gain based on the ambient illumination sensed by the electrically coupled horizontal cell network (Xin & Bloomfield, 1999). Consequently, signal integration over distances beyond the size of a horizontal cell dendritic tree strongly relies on the low-resistance gap junctional coupling of the horizontal network. In a broader sense, global signal processing can range from integrating the input from a few cones by a single horizontal cell to processing input from large cone populations by multiple electrically coupled horizontal cells (Chapot *et al.*, 2017)

In contrast, recent findings indicate that horizontal cells may also play a role in local signal processing by providing individual cones with “customized” feedback at the level of a single horizontal cell dendrite. Evidence for this comes from a study by Jackman *et al.*,2011, suggesting that horizontal cells have the ability to provide (excitatory) feedback locally to restore the cones dynamic range lost by inhibitory feedback.

However, the mechanism by which horizontal cells simultaneously achieve global and local signaling remains uncertain. In this context, Behrens *et al.,* 2022 propose that horizontal cells possess two synaptic strata that allow for simultaneous local feedback and global feedforward signaling by horizontal cells. In their study, the researchers reconstructed the circuitry of mouse horizontal cells based on five reconstructed horizontal cells within the outer retina. They

identified a second synaptic layer composed of short segments of increased dendritic diameter termed “bulbs” located on the primary dendrites of horizontal cells. These bulbs form GABAergic synapses with bipolar cells. Therefore, while the thin distal horizontal cell dendrite seems specialized for local cone-specific feedback, the bulbs are particularly suited for lateral inhibition. The GABAergic bulb synapse could transmit global signals not only to other horizontal cells but also to bipolar cells, potentially contributing to the center-surround antagonistic receptive field of these cells (Behrens *et al.*, 2022)

On the other hand, horizontal cells also contribute to the center-surround receptive fields of ganglion cells. In this context, a different study employed an alternative approach to analyze the influence of horizontal cells on ganglion cell signaling independent of feedback mechanisms in the outer retina and without manipulating signal transmission pharmacologically. The researchers developed a transgenic mouse line lacking the GluA2 and GluA4 subunits from AMPA receptors within horizontal cells, making them unresponsive to glutamatergic signals from photoreceptors. Consequently, feedback from horizontal cells was no longer modulated by light (Stroh *et al.*, 2018). Subsequently, the researchers recorded light responses in transient OFF-α retinal ganglion cells in wild-type and transgenic mouse lines. The outcome of this study suggests that the receptive fields of these cells were significantly altered in the transgenic mouse, where transient OFF-α retinal ganglion cells showed a substantial decrease in the spike rate and remained unresponsive to high temporal frequencies or low contrasts, indicating a compressed dynamic range. Additionally, the receptive field surround of the transient OFF-α retinal ganglion cells was substantially altered. These results strongly imply that horizontal cells adjust the dynamic range of ganglion cells and substantially contribute to shaping their receptive fields (Stroh *et al.*, 2018).

In a recent study from Gupta *et al.*, 2023, the researchers developed a model based on predictive coding (Srinivasan *et al.*, 1982) adapted to natural statistics of the mouse vision. Subsequently, the authors established a system to record and characterize the receptive fields of thousands of cell ganglion cells in a single retina. Their findings highlighted systematic variations of the ganglion cell receptive fields across the dorsoventral axes of the retina. In the dorsal retina, ganglion cells receptive fields showed less surround strength and larger center-size organization. In contrast, in the ventral retina, stronger surrounds and smaller center-size organization were observed (Gupta *et al.*, 2023). In a similar context, the dimensions of horizontal cell receptive fields vary based on the spatial detail of the stimulus. Therefore, the

size of horizontal cells receptive fields changes in response to the adaptational state of the retina and variations in background illumination (Mangel & Dowling, 1985; Yang *et al.,* 1988a; Tornqvist *et al.,* 1988; Baldridge & Ball, 1991). Moreover, given the role of horizontal cells in gain control and shaping the receptive fields of bipolar cells and ganglion cells, the outcomes of the investigations mentioned above align with the findings found in this study. In this context, horizontal cells form extensive networks via gap junctions, specifically through Cx57. Consequently, their receptive fields are much larger than their dendritic fields. Consequently, the spatial extent of the feedback signal to cones is primarily determined by the coupling strength in the horizontal cell network.

In this context, Figure 6 illustrates a gradient in horizontal cell coupling strength across the dorsoventral axes of the retina, which may be potentially attributed to the Cx57 gradient illustrated in Figure 4. The variations in coupling strength observed across the dorsoventral axes of the retina may be reflected in the receptive fields of downstream neurons, such as ganglion cells. These observations are likely consistent with the Gupta *et al*., 2023 findings. In their study, the authors found larger receptive field center sizes and decreased surround strength in ganglion cells in the dorsal retina. Conversely, ganglion cells in the ventral retina exhibited smaller center sizes and increased surround strength. However, the question remains: How do the outcomes presented in this study align with those of Gupta *et al.,* 2023? Larger receptive fields gather more information as they receive input from more cones, and this input is conveyed by more synapses. However, the extensive coupling between horizontal cells, given by the opening of gap junctions, results in a drop in input resistance and consequently creates a shunt in the current, reducing the local feedback from horizontal cells to photoreceptors (Pandarinath *et al.,* 2010). Consequently, lateral inhibition is reduced, and this is reflected in less surround strength in ganglion cells. However, if horizontal cells are also coupled in the ventral retina, why do ganglion cell receptive fields exhibit more strength surround and smaller center size? When gap junctions are closed, and horizontal cells are uncoupled (or less coupled), the input resistance increases, thereby strengthening horizontal cell signals and producing more robust feedback to photoreceptors (Pandarinath *et al.,* 2010). Therefore, since the strength of horizontal cell coupling is reduced in the ventral retina and fewer cells are coupled, the current is not shunted to the same extent as in the dorsal retina. Consequently, the input resistance in the ventral retina is higher than the dorsal retina, leading to a stronger lateral inhibition contributing to higher surround strength and smaller center sizes in ganglion cells receptive fields.

In this context, it has been described that stronger surround strength aids in better contrast discrimination. Hence, considering that the ventral retina is oriented toward the sky, it would be advantageous to have improved vision acuity on the ventral side to assist small animals in detecting potential predators approaching from above. Conversely, as the dorsal retina is directed toward the ground, perhaps sharp vision is not as crucial because mice can rely on other senses, such as whisking or smelling.

10. Outlook

To investigate further the functionality of horizontal cells within different regions of the retina, it would be interesting to measure light responses of cones in whole-mount preparations across the dorsoventral axes of the retina to analyze whether the feedback from horizontal cells changes across different areas of the retina. Similarly, exploring bipolar cell light responses across the dorsoventral axes of the retina could enhance our comprehension of the feedforward signaling from horizontal cells. The outcome of these experiments has the potential to contribute significantly to our understanding of how horizontal cells shape the receptive fields of upstream and downstream neurons across different retinal areas.

Conversely, intracellular injections and subsequent reconstruction of horizontal cells located within the hotspot could be employed to investigate whether there are differences in the morphology or connectivity of these cells compared to other regions. This investigation can potentially uncover specialized functions or adaptations unique to the horizontal cell ventral hotspot. Moreover, the analysis of light responses originating from cells within the hotspot could provide insights into whether these cells process visual information differentially across the retina in response to varying light conditions and natural scenes.

In summary, the proposed approaches carry the potential to discover novel insights into the functional differences and adaptations of horizontal cells in diverse retinal regions, particularly within the hotspot. Nevertheless, it is imperative to acknowledge the challenges intrinsic to this research, including the technical complexities associated with whole-mount preparations and cell injections. Success in this research could enhance our comprehension of retinal function and adaptation, thereby potentially improving our understanding of different visual processing across the retina.

11. Conclusion

This study reveals a novel gradient of horizontal cells within the mouse retina, where their distribution showcases distinct densities in the dorsal and ventral regions, with lower and higher densities on the dorsal and ventral sides, respectively. Interestingly, the mentioned gradient seems to overlap with the S-opsin transition zone. Moreover, a hotspot of horizontal cells was identified in the ventral part of the retina, which may reflect a retinal specialization.

Of particular interest, the morphometric analysis revealed that horizontal cells in the dorsal retina possess broader dendritic fields, contrasting with their counterparts in the ventral retina, which exhibit smaller dendritic trees. Nevertheless, despite these variations, horizontal cells are not different from each other in terms of branching pattern or number of dendritic tips.

Moreover, the data presented in this study provides compelling evidence of increased electrical coupling among horizontal cells located in the dorsal retina, which may be attributed to a higher Cx57 expression in that area. These findings contribute to a better understanding of how horizontal cells spatial scales and overlapping synaptic gradients provide modulatory feedback to postsynaptic cells and how they contribute downstream to visual processing, thus being able to regulate specific functions within the retina.

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13. References

- Ahnelt, P., & Kolb, H. (1994). Horizontal cells and cone photoreceptors in primate retina: a Golgi-light microscopic study of spectral connectivity. The Journal of comparative neurology, 343(3), 387–405. https://doi.org/10.1002/cne.903430305

- Andrade-da-Costa, B. L., Pessoa, V. F., Bousfield, J. D., & Clarke, R. J. (1989). Ganglion cell size and distribution in the retina of the two-toed sloth (Choloepus didactylus L.). Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas, 22(2), 233–236.

- Applebury, M. L., Antoch, M. P., Baxter, L. C., Chun, L. L., 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

- Arshavsky, V. Y., Lamb, T. D., & Pugh, E. N., Jr (2002). G proteins and phototransduction. Annual review of physiology, 64, 153–187. https://doi.org/10.1146/annurev.physiol.64.082701.102229

- Baden, T., Schubert, T., Chang, L., Wei, T., Zaichuk, M., Wissinger, B., & Euler, T. (2013). A tale of two retinal domains: near-optimal sampling of achromatic contrasts in natural scenes through asymmetric photoreceptor distribution. Neuron, 80(5), 1206–1217. https://doi.org/10.1016/j.neuron.2013.09.030

- Baden, T., Euler, T., & Berens, P. (2020). Understanding the retinal basis of vision across species. Nature reviews. Neuroscience, 21(1), 5–20. https://doi.org/10.1038/s41583-019-0242-1
- Baden T. (2020). Vertebrate vision: Lessons from non-model species. Seminars in cell & developmental biology, 106, 1–4. https://doi.org/10.1016/j.semcdb.2020.05.028

- Baldridge, W. H., & Ball, A. K. (1991). Background illumination reduces horizontal cell receptive-field size in both normal and 6-hydroxydopamine-lesioned goldfish retinas. Visual Neuroscience, 7(5), 441–450. https://doi.org/10.1017/s0952523800009731

- Barrantes-Freer, A., Renovanz, M., Eich, M., Braukmann, A., Sprang, B., Spirin, P., Pardo, L. A., Giese, A., & Kim, E. L. (2015). CD133 Expression Is Not Synonymous to Immunoreactivity for AC133 and Fluctuates throughout the Cell Cycle in Glioma Stem-Like Cells. PloS one, 10(6), e0130519. https://doi.org/10.1371/journal.pone.0130519

- BARLOW, H. B., FITZHUGH, R., & KUFFLER, S. W. (1957). Change of organization in the receptive fields of the cat's retina during dark adaptation. The Journal of Physiology, 137(3), 338–354. https://doi.org/10.1113/jphysiol.1957.sp005817

- Barnes, S., & Bui, Q. (1991). Modulation of calcium-activated chloride current via pH-induced changes of calcium channel properties in cone photoreceptors. The Journal of neuroscience : the official journal of the Society for Neuroscience, 11(12), 4015–4023. https://doi.org/10.1523/JNEUROSCI.11-12-04015.1991

- Barnes, S., Merchant, V., & Mahmud, F. (1993). Modulation of transmission gain by protons at the photoreceptor output synapse. Proceedings of the National Academy of Sciences of the United States of America, 90(21), 10081–10085. https://doi.org/10.1073/pnas.90.21.10081

- Barnes, S., Grove, J. C. R., McHugh, C. F., Hirano, A. A., & Brecha, N. C. (2020). Horizontal Cell Feedback to Cone Photoreceptors in Mammalian Retina: Novel Insights From the GABA-pH Hybrid Model. Frontiers in cellular neuroscience, 14, 595064. https://doi.org/10.3389/fncel.2020.595064

- Baylor, D. A., Nunn, B. J., & Schnapf, J. L. (1987). Spectral sensitivity of cones of the monkey Macaca fascicularis. The Journal of Physiology, 390, 145–160. https://doi.org/10.1113/jphysiol.1987.sp016691

- Beckwith-Cohen, B., Holzhausen, L. C., Wang, T. M., Rajappa, R., & Kramer, R. H. (2019). Localizing Proton-Mediated Inhibitory Feedback at the Retinal Horizontal Cell-Cone Synapse with Genetically-Encoded pH Probes. The Journal of neuroscience : the official journal of the Society for Neuroscience, 39(4), 651–662. https://doi.org/10.1523/JNEUROSCI.1541-18.2018

- Behrens, C., Yadav, S. C., Korympidou, M. M., Zhang, Y., Haverkamp, S., Irsen, S., Schaedler, A., Lu, X., Liu, Z., Lause, J., St-Pierre, F., Franke, K., Vlasits, A., Dedek, K., Smith, R. G., Euler, T., Berens, P., & Schubert, T. (2022). Retinal horizontal cells use different synaptic sites for global feedforward and local feedback signaling. Current biology : CB, 32(3), 545–558.e5. https://doi.org/10.1016/j.cub.2021.11.055

- Bleckert, A., Schwartz, G. W., Turner, M. H., Rieke, F., & Wong, R. O. (2014). Visual space is represented by nonmatching topographies of distinct mouse retinal ganglion cell types. Current biology: CB, 24(3), 310–315. https://doi.org/10.1016/j.cub.2013.12.020

- Bloomfield, S. A., & Völgyi, B. (2009). The diverse functional roles and regulation of neuronal gap junctions in the retina. Nature reviews. Neuroscience, 10(7), 495–506. https://doi.org/10.1038/nrn2636

- Bloomfield, S. A., Xin, D., & Persky, S. E. (1995). A comparison of receptive field and tracer coupling size of horizontal cells in the rabbit retina. Visual Neuroscience, 12(5), 985–999. https://doi.org/10.1017/s0952523800009524

- Bosone, C., Andreu, A., & Echevarria, D. (2016). GAP junctional communication in brain secondary organizers. Development, growth & differentiation, 58(5), 446–455. https://doi.org/10.1111/dgd.12297

- Boycott, B. B., Peichl, L., & Wässle, H. (1978). Morphological types of horizontal cell in the retina of the domestic cat. Proceedings of the Royal Society of London. Series B, Biological sciences, 203(1152), 229–245. https://doi.org/10.1098/rspb.1978.0103

- Boycott B. B. (1988). Horizontal cells of mammalian retinae. Neuroscience research. Supplement: the official journal of the Japan Neuroscience Society, 8, S97–S111. https://doi.org/10.1016/0921-8696(88)90010-2

- Burger, C. A., Jiang, D., Mackin, R. D., & Samuel, M. A. (2021). Development and maintenance of vision's first synapse. Developmental biology, 476, 218–239. https://doi.org/10.1016/j.ydbio.2021.04.001

- Burns, M. E., & Baylor, D. A. (2001). Activation, deactivation, and adaptation in vertebrate photoreceptor cells. Annual review of neuroscience, 24, 779–805. https://doi.org/10.1146/annurev.neuro.24.1.779

- Burns, M. E., & Arshavsky, V. Y. (2005). Beyond counting photons: trials and trends in vertebrate visual transduction. Neuron, 48(3), 387–401. https://doi.org/10.1016/j.neuron.2005.10.014

- Burns M. E. (2010). Deactivation mechanisms of rod phototransduction: the Cogan lecture. Investigative ophthalmology & visual science, 51(3), 1282–1288. https://doi.org/10.1167/iovs.09-4366

- Breuninger, T., Puller, C., Haverkamp, S., & Euler, T. (2011). Chromatic bipolar cell pathways in the mouse retina. The Journal of neuroscience: the official journal of the Society for Neuroscience, 31(17), 6504–6517. https://doi.org/10.1523/JNEUROSCI.0616-11.2011

- Bringmann, A., Syrbe, S., Görner, K., Kacza, J., Francke, M., Wiedemann, P., & Reichenbach, A. (2018). The primate fovea: Structure, function and development. Progress in retinal and eye research, 66, 49–84. https://doi.org/10.1016/j.preteyeres.2018.03.006

- Byzov A. L. (1975). Vzaimodeĭstvie mezhdu gorizontal'nymi kletkami setchatki cherepakhi [Interaction between the horizontal cells of the turtle retina]. Neirofiziologiia = Neurophysiology, 7(3), 279–286.

- Byzov, A. L., & Shura-Bura, T. M. (1986). Electrical feedback mechanism in the processing of signals in the outer plexiform layer of the retina. Vision research, 26(1), 33–44. https://doi.org/10.1016/0042-6989(86)90069-6

- Camerino, M. J., Engerbretson, I. J., Fife, P. A., Reynolds, N. B., Berria, M. H., Doyle, J. R., Clemons, M. R., Gencarella, M. D., Borghuis, B. G., & Fuerst, P. G. (2021). OFF bipolar cell density varies by subtype, eccentricity, and along the dorsal ventral axis in the mouse retina. The Journal of Comparative Neurology, 529(8), 1911–1925. https://doi.org/10.1002/cne.25064

- Chang, L., Breuninger, T., & Euler, T. (2013). Chromatic coding from cone-type unselective circuits in the mouse retina. Neuron, 77(3), 559–571. https://doi.org/10.1016/j.neuron.2012.12.012

- Chapot, C. A., Euler, T., & Schubert, T. (2017). How do horizontal cells 'talk' to cone photoreceptors? Different levels of complexity at the cone-horizontal cell synapse. The Journal of Physiology, 595(16), 5495–5506. https://doi.org/10.1113/JP274177

- 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. Scientific reports, 7(1), 5540. https://doi.org/10.1038/s41598-017-05543-2

- Choi, H. J., Ribelayga, C. P., & Mangel, S. C. (2012). Cut-loading: a useful tool for examining the extent of gap junction tracer coupling between retinal neurons. Journal of visualized experiments: JoVE, (59), 3180. https://doi.org/10.3791/3180

- Collin, S.P. and Partridge, J.C. (1996), Retinal specializations in the eyes of deep-sea teleosts. Journal of Fish Biology, 49: 157-174. https://doi.org/10.1111/j.1095-8649.1996.tb06073.x

- Collin S. P. (2008). A web-based archive for topographic maps of retinal cell distribution in vertebrates. Clinical & experimental optometry, 91(1), 85–95. https://doi.org/10.1111/j.1444-0938.2007.00228.x

- Connors, B. W., & Long, M. A. (2004). Electrical synapses in the mammalian brain. Annual review of neuroscience, 27, 393–418. https://doi.org/10.1146/annurev.neuro.26.041002.131128

- Dacheux, R. F., & Miller, R. F. (1981). An intracellular electrophysiological study of the ontogeny of functional synapses in the rabbit retina. I. Receptors, horizontal, and bipolar cells. The Journal of Comparative Neurology, 198(2), 307–326. https://doi.org/10.1002/cne.901980209

- Dacheux, R. F., & Raviola, E. (1982). Horizontal cells in the retina of the rabbit. The Journal of neuroscience: the official journal of the Society for Neuroscience, 2(10), 1486–1493. https://doi.org/10.1523/JNEUROSCI.02-10-01486.1982

- Daniels, B. A., & Baldridge, W. H. (2011). The light-induced reduction of horizontal cell receptive field size in the goldfish retina involves nitric oxide. Visual Neuroscience, 28(2), 137–144. https://doi.org/10.1017/S0952523810000490

- Detwiler, P. B., & Gray-Keller, M. P. (1996). The mechanisms of vertebrate light adaptation: speeded recovery versus slowed activation. Current opinion in neurobiology, 6(4), 440–444. https://doi.org/10.1016/s0959-4388(96)80047-x

- Davenport, C. M., Detwiler, P. B., & Dacey, D. M. (2008). Effects of pH buffering on horizontal and ganglion cell light responses in primate retina: evidence for the proton hypothesis of surround formation. The Journal of neuroscience : the official journal of the Society for Neuroscience, 28(2), 456–464. https://doi.org/10.1523/JNEUROSCI.2735-07.2008

- De Franceschi, G., Vivattanasarn, T., Saleem, A. B., & Solomon, S. G. (2016). Vision Guides Selection of Freeze or Flight Defense Strategies in Mice. Current biology: CB, 26(16), 2150–2154. https://doi.org/10.1016/j.cub.2016.06.006

- Denman, D. J., Luviano, J. A., Ollerenshaw, D. R., Cross, S., Williams, D., Buice, M. A., Olsen, S. R., & Reid, R. C. (2018). Mouse color and wavelength-specific luminance contrast sensitivity are non-uniform across visual space. eLife, 7, e31209. https://doi.org/10.7554/eLife.31209
- Dong, C. J., & McReynolds, J. S. (1991). The relationship between light, dopamine release and horizontal cell coupling in the mudpuppy retina. The Journal of physiology, 440, 291–309. https://doi.org/10.1113/jphysiol.1991.sp018709

- Dorgau, B., Herrling, R., Schultz, K., Greb, H., Segelken, J., Ströh, S., Bolte, P., Weiler, R., Dedek, K., & Janssen-Bienhold, U. (2015). Connexin50 couples axon terminals of mouse

horizontal cells by homotypic gap junctions. The Journal of Comparative Neurology, 523(14), 2062–2081. https://doi.org/10.1002/cne.23779

- Dowling, J. E., & Werblin, F. S. (1969). Organization of retina of the mudpuppy, Necturus maculosus. I. Synaptic structure. Journal of Neurophysiology, 32(3), 315–338. https://doi.org/10.1152/jn.1969.32.3.315

- Dräger, U. C., & Olsen, J. F. (1981). Ganglion cell distribution in the retina of the mouse. Investigative ophthalmology & visual science, 20(3), 285–293.

- Drinnenberg, A., Franke, F., Morikawa, R. K., Jüttner, J., Hillier, D., Hantz, P., Hierlemann, A., Azeredo da Silveira, R., & Roska, B. (2018). How Diverse Retinal Functions Arise from Feedback at the First Visual Synapse. Neuron, 99(1), 117–134.e11. https://doi.org/10.1016/j.neuron.2018.06.001

- Duda, S., Block, C.T., Pradhan, D.R, Arzhangnia, Y., Greschner, M., Puller, C. (2023). Spatial distribution and functional integration of displaced ipRGCs. bioRxiv. 2023 Sept 5. 556383. doi: https://doi.org/10.1101/2023.09.05.556383

- Fahrenfort, I., Klooster, J., Sjoerdsma, T., & Kamermans, M. (2005). The involvement of glutamate-gated channels in negative feedback from horizontal cells to cones. Progress in brain research, 147, 219–229. https://doi.org/10.1016/S0079-6123(04)47017-4

- Gaynes, J. A., Budoff, S. A., Grybko, M. J., Hunt, J. B., & Poleg-Polsky, A. (2022). Classical center-surround receptive fields facilitate novel object detection in retinal bipolar cells. Nature communications, 13(1), 5575. https://doi.org/10.1038/s41467-022-32761-8
- Gerl, E.J., Morris, M.R. The Causes and Consequences of Color Vision. Evo Edu Outreach 1, 476–486 (2008). https://doi.org/10.1007/s12052-008-0088-x

- Gouras, P., & Ekesten, B. (2004). Why do mice have ultra-violet vision? Experimental eye research, 79(6), 887–892. https://doi.org/10.1016/j.exer.2004.06.031
- Grove, J. C. R., Hirano, A. A., de Los Santos, J., McHugh, C. F., Purohit, S., Field, G. D., Brecha, N. C., & Barnes, S. (2019). Novel hybrid action of GABA mediates inhibitory

feedback in the mammalian retina. PLoS biology, 17(4), e3000200. https://doi.org/10.1371/journal.pbio.3000200

- Grünert, U., & Martin, P. R. (2020). Cell types and cell circuits in human and non-human primate retina. Progress in retinal and eye research, 100844. Advanced online publication. https://doi.org/10.1016/j.preteyeres.2020.100844

- Gupta, D., Młynarski, W., Sumser, A., Symonova, O., Svatoň, J., & Joesch, M. (2023). Panoramic visual statistics shape retina-wide organization of receptive fields. Nature Neuroscience, 26(4), 606–614. https://doi.org/10.1038/s41593-023-01280-0

- Hack, I., & Peichl, L. (1999). Horizontal cells of the rabbit retina are non-selectively connected to the cones. The European journal of neuroscience, 11(7), 2261–2274. https://doi.org/10.1046/j.1460-9568.1999.00647.x

- HARTLINE, H. K., WAGNER, H. G., & RATLIFF, F. (1956). Inhibition in the eye of Limulus. The Journal of general physiology, 39(5), 651–673. https://doi.org/10.1085/jgp.39.5.651

- HARTLINE, H. K., & RATLIFF, F. (1957). Inhibitory interaction of receptor units in the eye of Limulus. The Journal of general physiology, 40(3), 357–376. https://doi.org/10.1085/jgp.40.3.357

- HarveyJr, L.O. Vision, Psychology of.Neil J. Smelser, Paul B. Baltes, editors. International Encyclopedia of the Social & Behavioral Sciences, Pergamon, 2001, Pages 16237-16246 https://doi.org/10.1016/B0-08-043076-7/01468-6

- Haverkamp, S., Grünert, U., & Wässle, H. (2000). The cone pedicle, a complex synapse in the retina. Neuron, 27(1), 85–95. https://doi.org/10.1016/s0896-6273(00)00011-8

- He, S., Weiler, R., & Vaney, D. I. (2000). Endogenous dopaminergic regulation of horizontal cell coupling in the mammalian retina. Journal of Comparative Neurology, 418(1), 33-40. https://doi.org/10.1002/(SICI)1096-9861(20000228)418:1<33::AID-CNE3>3.0.CO;2-J

- Hendrickson A. 2005. Organization of the adult primate fovea. In Macular Degeneration, ed. PL Penfold, JM Provis, pp. 1–23. Berlin: Springer

- Heukamp, A. S., Warwick, R. A., & Rivlin-Etzion, M. (2020). Topographic Variations in Retinal Encoding of Visual Space. Annual review of vision science, 6, 237–259. https://doi.org/10.1146/annurev-vision-121219-081831

- Hirano, A. A., Liu, X., Boulter, J., Grove, J., Pérez de Sevilla Müller, L., Barnes, S., & Brecha, N. C. (2016). Targeted Deletion of Vesicular GABA Transporter from Retinal Horizontal Cells Eliminates Feedback Modulation of Photoreceptor Calcium Channels. eNeuro, 3(2), ENEURO.0148-15.2016. https://doi.org/10.1523/ENEURO.0148-15.2016

- Hirano, A. A., Vuong, H. E., Kornmann, H. L., Schietroma, C., Stella, S. L., Jr, Barnes, S., & Brecha, N. C. (2020). Vesicular Release of GABA by Mammalian Horizontal Cells Mediates Inhibitory Output to Photoreceptors. Frontiers in cellular neuroscience, 14, 600777. https://doi.org/10.3389/fncel.2020.600777

- Hirasawa, H., & Kaneko, A. (2003). pH changes in the invaginating synaptic cleft mediate feedback from horizontal cells to cone photoreceptors by modulating Ca2+ channels. The Journal of general physiology, 122(6), 657–671. https://doi.org/10.1085/jgp.200308863

- 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. The European journal of neuroscience, 19(10), 2633–2640. https://doi.org/10.1111/j.0953-816X.2004.03360.x

- Horikawa, K., & Armstrong, W. E. (1988). A versatile means of intracellular labeling: injection of biocytin and its detection with avidin conjugates. Journal of neuroscience methods, 25(1), 1–11. https://doi.org/10.1016/0165-0270(88)90114-8
- Hughes A. (1975). A quantitative analysis of the cat retinal ganglion cell topography. The Journal of Comparative Neurology, 163(1), 107–128. https://doi.org/10.1002/cne.901630107

- Hughes, A. (1977). The Topography of Vision in Mammals of Contrasting Life Style: Comparative Optics and Retinal Organisation. In: Crescitelli, F. (eds) The Visual System in Vertebrates. Handbook of Sensory Physiology, vol 7 / 5. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-66468-7_11

- Hughes, S., Watson, T. S., Foster, R. G., Peirson, S. N., & Hankins, M. W. (2013). Nonuniform distribution and spectral tuning of photosensitive retinal ganglion cells of the mouse retina. Current biology: CB, 23(17), 1696–1701. https://doi.org/10.1016/j.cub.2013.07.010

- Hunt, D. M., & Peichl, L. (2014). S cones: Evolution, retinal distribution, development, and spectral sensitivity. Visual Neuroscience, 31(2), 115–138. https://doi.org/10.1017/S0952523813000242

- Jackman, S. L., Babai, N., Chambers, J. J., Thoreson, W. B., & Kramer, R. H. (2011). A positive feedback synapse from retinal horizontal cells to cone photoreceptors. PLoS biology, 9(5), e1001057. https://doi.org/10.1371/journal.pbio.1001057

- Janssen-Bienhold, U., Trümpler, J., Hilgen, G., Schultz, K., Müller, L. P., Sonntag, S., Dedek, K., Dirks, P., Willecke, K., & Weiler, R. (2009). Connexin57 is expressed in dendro-dendritic and axo-axonal gap junctions of mouse horizontal cells and its distribution is modulated by light. The Journal of Comparative Neurology, 513(4), 363–374. https://doi.org/10.1002/cne.21965

- Jeon, C. J., Strettoi, E., & Masland, R. H. (1998). The major cell populations of the mouse retina. The Journal of neuroscience: the official journal of the Society for Neuroscience, 18(21), 8936–8946. https://doi.org/10.1523/JNEUROSCI.18-21-08936.1998

- Kaneko A. (1971). Electrical connexions between horizontal cells in the dogfish retina. The Journal of Physiology, 213(1), 95–105. https://doi.org/10.1113/jphysiol.1971.sp009370

- Kaneko, A., & Stuart, A. E. (1984). Coupling between horizontal cells in the carp retina revealed by diffusion of Lucifer yellow. Neuroscience letters, 47(1), 1–7. https://doi.org/10.1016/0304-3940(84)90377-x.

- Kaneko, A., & Tachibana, M. (1986). Effects of gamma-aminobutyric acid on isolated cone photoreceptors of the turtle retina. The Journal of physiology, 373, 443–461. https://doi.org/10.1113/jphysiol.1986.sp016057

- Kim, U. S., Mahroo, O. A., Mollon, J. D., & Yu-Wai-Man, P. (2021). Retinal Ganglion Cells-Diversity of Cell Types and Clinical Relevance. Frontiers in neurology, 12, 661938. https://doi.org/10.3389/fneur.2021.661938

- 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. Proceedings of the National Academy of Sciences of the United States of America, 107(1), 332–337. https://doi.org/10.1073/pnas.0912730107

- Kramer, R. H., & Davenport, C. M. (2015). Lateral Inhibition in the Vertebrate Retina: The Case of the Missing Neurotransmitter. PLoS biology, 13(12), e1002322. https://doi.org/10.1371/journal.pbio.1002322

- Kumar, N. M., & Gilula, N. B. (1996). The gap junction communication channel. Cell, 84(3), 381–388. https://doi.org/10.1016/s0092-8674(00)81282-9

- Laitinen, O. H., Hytönen, V. P., Nordlund, H. R., & Kulomaa, M. S. (2006). Genetically engineered avidins and streptavidins. Cellular and molecular life sciences: CMLS, 63(24), 2992–3017. https://doi.org/10.1007/s00018-006-6288-z

- Lamb T. D. (1995). Photoreceptor spectral sensitivities: common shape in the long-wavelength region. Vision Research, 35(22), 3083–3091. https://doi.org/10.1016/0042-6989(95)00114-f
- Levick W. R. (1967). Receptive fields and trigger features of ganglion cells in the visual streak of the rabbits retina. The Journal of Physiology, 188(3), 285–307. https://doi.org/10.1113/jphysiol.1967.sp008140

- Li, A., Cho, J. H., Reid, B., Tseng, C. C., He, L., Tan, P., Yeh, C. Y., Wu, P., Li, Y., Widelitz, R. B., Zhou, Y., Zhao, M., Chow, R. H., & Chuong, C. M. (2018). Calcium oscillations coordinate feather mesenchymal cell movement by SHH dependent modulation of gap junction networks. Nature communications, 9(1), 5377. https://doi.org/10.1038/s41467-018-07661-5

- Linberg, K. A., Suemune, S., & Fisher, S. K. (1996). Retinal neurons of the California ground squirrel, Spermophilus beecheyi: a Golgi study. The Journal of Comparative Neurology, 365(2), 173–216. https://doi.org/10.1002/(SICI)1096-9861(19960205)365:2<173::AID-CNE1>3.0.CO;2-2

- Loewenstein W. R. (1981). Junctional intercellular communication: the cell-to-cell membrane channel. Physiological reviews, 61(4), 829–913. https://doi.org/10.1152/physrev.1981.61.4.829

- Luo, D.G., Su, C.Y., and Yau, K.W. (2009). Photoreceptors: physiology. Encycl. Neurosci. 677–686. https://doi.org/10.1016/B978-008045046-9.00913-X

- Mahabadi N, Al Khalili Y. Neuroanatomy, Retina. [Updated 2023 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545310/
- Mangel, S. C., & Dowling, J. E. (1985). Responsiveness and receptive field size of carp horizontal cells are reduced by prolonged darkness and dopamine. Science (New York, N.Y.), 229(4718), 1107–1109. https://doi.org/10.1126/science.4035351
- Marr, D., & Hildreth, E. (1980). Theory of edge detection. Proceedings of the Royal Society of London. Series B, Biological sciences, 207(1167), 187–217. https://doi.org/10.1098/rspb.1980.0020

- Martin, E. A., Lasseigne, A. M., & Miller, A. C. (2020). Understanding the Molecular and Cell Biological Mechanisms of Electrical Synapse Formation. Frontiers in neuroanatomy, 14, 12. https://doi.org/10.3389/fnana.2020.00012

- Masland R. H. (2012). The neuronal organization of the retina. Neuron, 76(2), 266–280. https://doi.org/10.1016/j.neuron.2012.10.002

- 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. The Journal of comparative neurology, 502(6), 1123–1137. https://doi.org/10.1002/cne.21367

- Meyer, A., Yadav, S. C., & Dedek, K. (2018). Phenotyping of Gap-Junctional Coupling in the Mouse Retina. Methods in molecular biology (Clifton, N.J.), 1753, 249–259. https://doi.org/10.1007/978-1-4939-7720-8_17

- Mills, S. L., & Massey, S. C. (2000). A series of biotinylated tracers distinguishes three types of gap junction in retina. The Journal of neuroscience : the official journal of the Society for Neuroscience, 20(22), 8629–8636. https://doi.org/10.1523/JNEUROSCI.20-22-08629.2000

- Mitchell, C. K., Rowe-Rendleman, C. L., Ashraf, S., & Redburn, D. A. (1995). Calbindin immunoreactivity of horizontal cells in the developing rabbit retina. Experimental eye research, 61(6), 691–698. https://doi.org/10.1016/s0014-4835(05)80020-x

- Morgans, C. W., Zhang, J., Jeffrey, B. G., Nelson, S. M., Burke, N. S., Duvoisin, R. M., & Brown, R. L. (2009). TRPM1 is required for the depolarizing light response in retinal ON-bipolar cells. Proceedings of the National Academy of Sciences of the United States of America, 106(45), 19174–19178. https://doi.org/10.1073/pnas.0908711106

- Münch, T. A., da Silveira, R. A., Siegert, S., Viney, T. J., Awatramani, G. B., & Roska, B. (2009). Approach sensitivity in the retina processed by a multifunctional neural circuit. Nature Neuroscience, 12(10), 1308–1316. https://doi.org/10.1038/nn.2389

- Myles, W. E., & McFadden, S. A. (2022). Analytical methods for assessing retinal cell coupling using cut-loading. PloS one, 17(7), e0271744. https://doi.org/10.1371/journal.pone.0271744

- Nadal-Nicolás, F. M., Kunze, V. P., Ball, J. M., Peng, B. T., Krishnan, A., Zhou, G., Dong, L., & Li, W. (2020). True S-cones are concentrated in the ventral mouse retina and wired for color detection in the upper visual field. eLife, 9, e56840. https://doi.org/10.7554/eLife.56840

- Naka K. I. (1972). The horizontal cells. Vision Research, 12(4), 573–588. https://doi.org/10.1016/0042-6989(72)90153-8

- Nakajima, Y., Iwakabe, H., Akazawa, C., Nawa, H., Shigemoto, R., Mizuno, N., & Nakanishi, S. (1993). Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. The Journal of Biological Chemistry, 268(16), 11868–11873.

- Nakase, T., & Naus, C. C. (2004). Gap junctions and neurological disorders of the central nervous system. Biochimica et biophysica acta, 1662(1-2), 149–158. https://doi.org/10.1016/j.bbamem.2004.01.009

- Nawy S. (1999). The metabotropic receptor mGluR6 may signal through G(o), but not phosphodiesterase, in retinal bipolar cells. The Journal of neuroscience: the official journal of the Society for Neuroscience, 19(8), 2938–2944. https://doi.org/10.1523/JNEUROSCI.19-08-02938.1999

- Nemitz, L., Dedek, K., & Janssen-Bienhold, U. (2019). Rod Bipolar Cells Require Horizontal Cells for Invagination Into the Terminals of Rod Photoreceptors. Frontiers in cellular neuroscience, 13, 423. https://doi.org/10.3389/fncel.2019.00423

- Omri, S., Omri, B., Savoldelli, M., Jonet, L., Thillaye-Goldenberg, B., Thuret, G., Gain, P., Jeanny, J. C., Crisanti, P., & Behar-Cohen, F. (2010). The outer limiting membrane (OLM) revisited: clinical implications. Clinical ophthalmology (Auckland, N.Z.), 4, 183–195. https://doi.org/10.2147/opth.s5901

- Osorio, D., & Vorobyev, M. (2005). Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and color vision. Proceedings. Biological sciences, 272(1574), 1745–1752. https://doi.org/10.1098/rspb.2005.3156

- Pandarinath, C., Bomash, I., Victor, J. D., Prusky, G. T., Tschetter, W. W., & Nirenberg, S. (2010). A novel mechanism for switching a neural system from one state to another. Frontiers in computational neuroscience, 4, 2. https://doi.org/10.3389/fncom.2010.00002

- Peichl, L., & González-Soriano, J. (1994). Morphological types of horizontal cell in rodent retinae: a comparison of rat, mouse, gerbil, and guinea pig. Visual Neuroscience, 11(3), 501–517. https://doi.org/10.1017/s095252380000242x

- Peichl L, Sandmann D, Boycott BB (1998) Comparative anatomy and function of mammalian horizontal cells. In: Development and organization of the retina (Chalupa LM, Finlay BL, eds). New York: Plenum.

- Peichl L. (2005). Diversity of mammalian photoreceptor properties: adaptations to habitat and lifestyle? The anatomical record. Part A, Discoveries in molecular, cellular, and evolutionary biology, 287(1), 1001–1012. https://doi.org/10.1002/ar.a.20262

- Peichl L. Morphology of Interneurons: Horizontal Cells. In: Darlene A. Dartt, editor. Encyclopedia of the Eye, Vol 3. Oxford: Academic Press; 2010. pp. 74-82

- Pepperberg D. R. (2003). Bleaching desensitization: background and current challenges. Vision research, 43(28), 3011–3019. https://doi.org/10.1016/s0042-6989(03)00484-x

- Poché, R. A., Raven, M. A., Kwan, K. M., Furuta, Y., Behringer, R. R., & Reese, B. E. (2008). Somal positioning and dendritic growth of horizontal cells are regulated by interactions with homotypic neighbors. The European journal of neuroscience, 27(7), 1607–1614. https://doi.org/10.1111/j.1460-9568.2008.06132.x

- Pogoda, K., Kameritsch, P., Retamal, M. A., & Vega, J. L. (2016). Regulation of gap junction channels and hemichannels by phosphorylation and redox changes: a revision. BMC cell biology, 17 Suppl 1(Suppl 1), 11. https://doi.org/10.1186/s12860-016-0099-3

- Polyak SL. 1941. The Retina. Chicago: Univ. Chicago Press

- Polyak SL. 1957. The Vertebrate Visual System. Chicago: Univ. Chicago Press

- Pottek, M., Schultz, K., & Weiler, R. (1997). Effects of nitric oxide on the horizontal cell network and dopamine release in the carp retina. Vision Research, 37(9), 1091–1102. https://doi.org/10.1016/s0042-6989(96)00298-2

- Pottek, M., & Weiler, R. (2000). Light-adaptive effects of retinoic acid on receptive field properties of retinal horizontal cells. The European journal of neuroscience, 12(2), 437–445. https://doi.org/10.1046/j.1460-9568.2000.00918.x

- Purves D, Augustine GJ, Fitzpatrick D, et al., editors. Neuroscience. 2nd edition. Sunderland (MA): Sinauer Associates; 2001. Phototransduction. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10806/

- Qiu, Y., Zhao, Z., Klindt, D., Kautzky, M., Szatko, K. P., Schaeffel, F., Rifai, K., Franke, K., Busse, L., & Euler, T. (2021). Natural environment statistics in the upper and lower visual field are reflected in mouse retinal specializations. Current biology: CB, 31(15), 3233–3247.e6. https://doi.org/10.1016/j.cub.2021.05.017

- Raven, M. A., Oh, E. C., Swaroop, A., & Reese, B. E. (2007). Afferent control of horizontal cell morphology revealed by genetic respecification of rods and cones. The Journal of neuroscience: the official journal of the Society for Neuroscience, 27(13), 3540–3547. https://doi.org/10.1523/JNEUROSCI.0372-07.2007

- Raven, M. A., Orton, N. C., Nassar, H., Williams, G. A., Stell, W. K., Jacobs, G. H., Bech-Hansen, N. T., & Reese, B. E. (2008). Early afferent signaling in the outer plexiform layer regulates the development of horizontal cell morphology. The Journal of Comparative Neurology, 506(5), 745–758. https://doi.org/10.1002/cne.21526

- Reese, B. E., Raven, M. A., & Stagg, S. B. (2005). Afferents and homotypic neighbors regulate horizontal cell morphology, connectivity, and retinal coverage. The Journal of neuroscience: the official journal of the Society for Neuroscience, 25(9), 2167–2175. https://doi.org/10.1523/JNEUROSCI.4876-04.2005

- Reese, B.E. (2008b). Mosaics, tiling, and coverage by retinal neurons. In The Senses: A Comprehensive Reference, Vision I, Vol. 1, ed. Masland, R.H. & Albright, T., pp. 439–456. Amsterdam: Elsevier.
- Rhim, I., Coello-Reyes, G., Ko, H. K., & Nauhaus, I. (2017). Maps of cone opsin input to mouse V1 and higher visual areas. Journal of Neurophysiology, 117(4), 1674–1682. https://doi.org/10.1152/jn.00849.2016

- Ribelayga, C., & Mangel, S. C. (2003). The absence of circadian clock regulation of horizontal cell gap junctional coupling reveals two dopamine systems in the goldfish retina. The Journal of Comparative Neurology, 467(2), 243–253. https://doi.org/10.1002/cne.10927
- Ribelayga, C., & Mangel, S. C. (2010). Identification of a circadian clock-controlled neural pathway in the rabbit retina. PloS one, 5(6), e11020. https://doi.org/10.1371/journal.pone.0011020

- Röhlich, P., van Veen, T., & Szél, A. (1994). Two different visual pigments in one retinal cone cell. Neuron, 13(5), 1159–1166. https://doi.org/10.1016/0896-6273(94)90053-1
- Saez, J. C., Berthoud, V. M., Branes, M. C., Martinez, A. D., & Beyer, E. C. (2003). Plasma membrane channels formed by connexins: their regulation and functions. Physiological reviews, 83(4), 1359–1400. https://doi.org/10.1152/physrev.00007.2003

- Schiviz, A. N., Ruf, T., Kuebber-Heiss, A., Schubert, C., & Ahnelt, P. K. (2008). Retinal cone topography of artiodactyl mammals: influence of body height and habitat. The Journal of Comparative Neurology, 507(3), 1336–1350. https://doi.org/10.1002/cne.21626
- 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. The European journal of neuroscience, 23(12), 3176–3186. https://doi.org/10.1111/j.1460-9568.2006.04848.x

- Shepherd, T. M., Thelwall, P. E., Stanisz, G. J., & Blackband, S. J. (2009). Aldehyde fixative solutions alter the water relaxation and diffusion properties of nervous tissue. Magnetic resonance in medicine, 62(1), 26–34. https://doi.org/10.1002/mrm.21977

- Schnapf, J. L., Kraft, T. W., & Baylor, D. A. (1987). Spectral sensitivity of human cone photoreceptors. Nature, 325(6103), 439–441. https://doi.org/10.1038/325439a0
- SHOLL D. A. (1953). Dendritic organization in the neurons of the visual and motor cortices of the cat. Journal of Anatomy, 87(4), 387–406.

- Skrzypek J. (1984). Electrical coupling between horizontal cell bodies in the tiger salamander retina. Vision Research, 24(7), 701–711. https://doi.org/10.1016/0042-6989(84)90211-6

- Söhl, G., Maxeiner, S., & Willecke, K. (2005). Expression and functions of neuronal gap junctions. Nature reviews. Neuroscience, 6(3), 191–200. https://doi.org/10.1038/nrn1627

- Sondereker, K. B., Stabio, M. E., Jamil, J. R., Tarchick, M. J., & Renna, J. M. (2018). Where You Cut Matters: A Dissection and Analysis Guide for the Spatial Orientation of the Mouse Retina from Ocular Landmarks. Journal of visualized experiments: JoVE, (138), 57861. https://doi.org/10.3791/57861
- Srinivasan, M. V., Laughlin, S. B., & Dubs, A. (1982). Predictive coding: a fresh view of inhibition in the retina. Proceedings of the Royal Society of London. Series B, Biological sciences, 216(1205), 427–459. https://doi.org/10.1098/rspb.1982.0085

- Stabio, M. E., Sondereker, K. B., Haghgou, S. D., Day, B. L., Chidsey, B., Sabbah, S., & Renna, J. M. (2018). A novel map of the mouse eye for orienting retinal topography in anatomical space. The Journal of Comparative Neurology, 526(11), 1749–1759. https://doi.org/10.1002/cne.24446

- Sterratt, D. C., Lyngholm, D., Willshaw, D. J., & Thompson, I. D. (2013). Standard anatomical and visual space for the mouse retina: computational reconstruction and transformation of flattened retinae with the Retistruct package. PLoS Computational Biology, 9(2), e1002921. https://doi.org/10.1371/journal.pcbi.1002921

- Ströh, S., Puller, C., Swirski, S., Hölzel, M. B., van der Linde, L. I. S., Segelken, J., Schultz, K., Block, C., Monyer, H., Willecke, K., Weiler, R., Greschner, M., Janssen-Bienhold, U., & Dedek, K. (2018). Eliminating Glutamatergic Input onto Horizontal Cells Changes the Dynamic Range and Receptive Field Organization of Mouse Retinal Ganglion Cells. The

Journal of neuroscience: the official journal of the Society for Neuroscience, 38(8), 2015–2028. https://doi.org/10.1523/JNEUROSCI.0141-17.2018

- Szatko, K. P., Korympidou, M. M., Ran, Y., Berens, P., Dalkara, D., Schubert, T., Euler, T., & Franke, K. (2020). Neural circuits in the mouse retina support color vision in the upper visual field. Nature communications, 11(1), 3481. https://doi.org/10.1038/s41467-020-17113-8

- Szél, A., Röhlich, P., Caffé, A. R., Juliusson, B., Aguirre, G., & Van Veen, T. (1992). Unique topographic separation of two spectral classes of cones in the mouse retina. The Journal of Comparative Neurology, 325(3), 327–342. https://doi.org/10.1002/cne.903250302

- Talukdar, S., Emdad, L., Das, S. K., & Fisher, P. B. (2022). GAP junctions: multifaceted regulators of neuronal differentiation. Tissue barriers, 10(1), 1982349. https://doi.org/10.1080/21688370.2021.1982349
- Tetenborg, S., Liss, V., Breitsprecher, L., Timonina, K., Kotova, A., Acevedo Harnecker, A. J., Yuan, C., Shihabeddin, E., Ariakia, F., Qin, G., Chengzhi, C., Dedek, K., Zoidl, G., Hensel, M., & O'Brien, J. (2023). Intraluminal docking of connexin 36 channels in the ER isolates mistrafficked protein. The Journal of Biological Chemistry, 299(11), 105282. https://doi.org/10.1016/j.jbc.2023.105282

- Thoreson, W. B., Babai, N., & Bartoletti, T. M. (2008). Feedback from horizontal cells to rod photoreceptors in vertebrate retina. The Journal of neuroscience: the official journal of the Society for Neuroscience, 28(22), 5691–5695. https://doi.org/10.1523/JNEUROSCI.0403-08.2008

- Thoreson, W. B., & Mangel, S. C. (2012). Lateral interactions in the outer retina. Progress in retinal and eye research, 31(5), 407–441. https://doi.org/10.1016/j.preteyeres.2012.04.003

- Tian, X., Cui, Z., Liu, S., Zhou, J., & Cui, R. (2021). Melanosome transport and regulation in development and disease. Pharmacology & therapeutics, 219, 107707. https://doi.org/10.1016/j.pharmthera.2020.107707

- Tornqvist, K., Yang, X. L., & Dowling, J. E. (1988). Modulation of cone horizontal cell activity in the teleost fish retina. III. Effects of prolonged darkness and dopamine on electrical coupling between horizontal cells. The Journal of neuroscience: the official journal of the Society for Neuroscience, 8(7), 2279–2288. https://doi.org/10.1523/JNEUROSCI.08-07-02279.1988

- Trenholm, S., & Baldridge, W. H. (2010). The effect of aminosulfonate buffers on the light responses and intracellular pH of goldfish retinal horizontal cells. Journal of neurochemistry, 115(1), 102–111. https://doi.org/10.1111/j.1471-4159.2010.06906.x

- Troy, J. B., Bohnsack, D. L., & Diller, L. C. (1999). Spatial properties of the cat X-cell receptive field as a function of mean light level. Visual Neuroscience, 16(6), 1089–1104. https://doi.org/10.1017/s0952523899166094

- Vaney D. I. (1991). Many diverse types of retinal neurons show tracer coupling when injected with biocytin or Neurobiotin. Neuroscience letters, 125(2), 187–190. https://doi.org/10.1016/0304-3940(91)90024-n

- Vaney D. I. (1993). The coupling pattern of axon-bearing horizontal cells in the mammalian retina. Proceedings. Biological Sciences, 252(1334), 93–101. https://doi.org/10.1098/rspb.1993.0051

- VanLeeuwen, M., Fahrenfort, I., Sjoerdsma, T., Numan, R., & Kamermans, M. (2009). Lateral gain control in the outer retina leads to potentiation of center responses of retinal neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience, 29(19), 6358–6366. https://doi.org/10.1523/JNEUROSCI.5834-08.2009

- Vaughn, M. J., & Haas, J. S. (2022). On the Diverse Functions of Electrical Synapses. Frontiers in cellular neuroscience, 16, 910015. https://doi.org/10.3389/fncel.2022.910015

- Vessey, J. P., Stratis, A. K., Daniels, B. A., Da Silva, N., Jonz, M. G., Lalonde, M. R., Baldridge, W. H., & Barnes, S. (2005). Proton-mediated feedback inhibition of presynaptic calcium channels at the cone photoreceptor synapse. The Journal of neuroscience : the official

journal of the Society for Neuroscience, 25(16), 4108–4117. https://doi.org/10.1523/JNEUROSCI.5253-04.2005

- VILLEGAS G. M. (1964). ULTRASTRUCTURE OF THE HUMAN RETINA. Journal of Anatomy, 98(Pt 4), 501–513.

- Völgyi, B., Kovács-Oller, T., Atlasz, T., Wilhelm, M., & Gábriel, R. (2013). Gap junctional coupling in the vertebrate retina: variations on one theme? Progress in retinal and eye research, 34, 1–18. https://doi.org/10.1016/j.preteyeres.2012.12.002

- Vroman, R., Klaassen, L. J., & Kamermans, M. (2013). Ephaptic communication in the vertebrate retina. Frontiers in human neuroscience, 7, 612. https://doi.org/10.3389/fnhum.2013.00612

- Vroman, R., Klaassen, L. J., Howlett, M. H., Cenedese, V., Klooster, J., Sjoerdsma, T., & Kamermans, M. (2014). Extracellular ATP hydrolysis inhibits synaptic transmission by increasing ph buffering in the synaptic cleft. PLoS biology, 12(5), e1001864. https://doi.org/10.1371/journal.pbio.1001864

- Walls, G. L. (1942). The vertebrate eye and its adaptive radiation. Cranbrook Institute of Science. https://doi.org/10.5962/bhl.title.7369

- Wang, T. M., Holzhausen, L. C., & Kramer, R. H. (2014). Imaging an optogenetic pH sensor reveals that protons mediate lateral inhibition in the retina. Nature neuroscience, 17(2), 262–268. https://doi.org/10.1038/nn.3627

- Warren, T. J., Van Hook, M. J., Tranchina, D., & Thoreson, W. B. (2016). Kinetics of Inhibitory Feedback from Horizontal Cells to Photoreceptors: Implications for an Ephaptic Mechanism. The Journal of neuroscience : the official journal of the Society for Neuroscience, 36(39), 10075–10088. https://doi.org/10.1523/JNEUROSCI.1090-16.2016

- Warwick, R. A., Kaushansky, N., Sarid, N., Golan, A., & Rivlin-Etzion, M. (2018). Inhomogeneous Encoding of the Visual Field in the Mouse Retina. Current biology: CB, 28(5), 655–665.e3. https://doi.org/10.1016/j.cub.2018.01.016

- Wässle, H., Peichl, L., & Boycott, B. B. (1978). Topography of horizontal cells in the retina of the domestic cat. Proceedings of the Royal Society of London. Series B, Biological sciences, 203(1152), 269–291. https://doi.org/10.1098/rspb.1978.0105

- Wässle, H., Puller, C., Müller, F., & Haverkamp, S. (2009). Cone contacts, mosaics, and territories of bipolar cells in the mouse retina. The Journal of neuroscience: the official journal of the Society for Neuroscience, 29(1), 106–117. https://doi.org/10.1523/JNEUROSCI.4442-08.2009

- Weiler, R., He, S., & Vaney, D. I. (1999). Retinoic acid modulates gap junctional permeability between horizontal cells of the mammalian retina. The European journal of neuroscience, 11(9), 3346–3350. https://doi.org/10.1046/j.1460-9568.1999.00799.x
- Weiler, R., Pottek, M., He, S., & Vaney, D. I. (2000). Modulation of coupling between retinal horizontal cells by retinoic acid and endogenous dopamine. Brain research. Brain research reviews, 32(1), 121–129. https://doi.org/10.1016/s0165-0173(99)00071-5

- Werblin F. S. (1974). Control of retinal sensitivity. II. Lateral interactions at the outer plexiform layer. The Journal of general physiology, 63(1), 62–87. https://doi.org/10.1085/jgp.63.1.62

- Wehrl, H. F., Bezrukov, I., Wiehr, S., Lehnhoff, M., Fuchs, K., Mannheim, J. G., Quintanilla-Martinez, L., Kohlhofer, U., Kneilling, M., Pichler, B. J., & Sauter, A. W. (2015). Assessment of murine brain tissue shrinkage caused by different histological fixatives using magnetic resonance and computed tomography imaging. Histology and histopathology, 30(5), 601–613. https://doi.org/10.14670/HH-30.601
- Werner, M., Chott, A., Fabiano, A., & Battifora, H. (2000). Effect of formalin tissue fixation and processing on immunohistochemistry. The American journal of surgical pathology, 24(7), 1016–1019. https://doi.org/10.1097/00000478-200007000-00014

- Wu, S. M., & Dowling, J. E. (1980). Effects of GABA and glycine on the distal cells of the cyprinid retina. Brain research, 199(2), 401–414. https://doi.org/10.1016/0006-8993(80)90697-6

- Yang, X. L., Tornqvist, K., & Dowling, J. E. (1988). Modulation of cone horizontal cell activity in the teleost fish retina. I. Effects of prolonged darkness and background illumination on light responsiveness. The Journal of neuroscience: the official journal of the Society for Neuroscience, 8(7), 2259–2268. https://doi.org/10.1523/JNEUROSCI.08-07-02259.1988

- Yin, L., Smith, R. G., Sterling, P., & Brainard, D. H. (2006). Chromatic properties of horizontal and ganglion cell responses follow a dual gradient in cone opsin expression. The Journal of neuroscience: the official journal of the Society for Neuroscience, 26(47), 12351–12361. https://doi.org/10.1523/JNEUROSCI.1071-06.2006

- Zhang, A. J., Jacoby, R., & Wu, S. M. (2011). Light- and dopamine-regulated receptive field plasticity in primate horizontal cells. The Journal of Comparative Neurology, 519(11), 2125–2134. https://doi.org/10.1002/cne.22604

- Zhang, Y., Kim, I. J., Sanes, J. R., & Meister, M. (2012). The most numerous ganglion cell type of the mouse retina is a selective feature detector. Proceedings of the National Academy of Sciences of the United States of America, 109(36), E2391–E2398. https://doi.org/10.1073/pnas.1211547109

- Zhou, J. Z., & Jiang, J. X. (2014). Gap junction and hemichannel-independent actions of connexins on cell and tissue functions--an update. FEBS letters, 588(8), 1186–1192. https://doi.org/10.1016/j.febslet.2014.01.001

14. Contributions/publications

14.1 Publications

1. Intralumenal docking of Cx36 channels in the ER isolates mis-trafficked protein

Stephan Tetenborg[1,*], Viktoria Liss[2,3,7], Leonhard Breitsprecher[2,3,7], Ksenia Timonina[4,7], Anna Kotova[4,7], Alejandra Jesús Acevedo Harnecker[5], Chunxu Yuan[5], Eyad Shihabeddin[1], Karin Dedek[5,6], Georg Zoidl[3], Michael Hensel[2,3] and John O`Brien[1,*].

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Authors' contributions

This study was designed by John O`Brien and Stephan Tetenborg. Chunxu Yuan conducted the cell culture and transient transfection of HEK cells. Subsequent confocal scanning and colocalization analysis were performed by Alejandra J. Acevedo Harnecker, who additionally contributed to the writing of the methods section and revision of the manuscript together with Karin Dedek and Chunxu Yuan. Figures were prepared by Stephan Tetenborg and John O`Brien. The first draft of the manuscript was written by Stephan Tetenborg, with all authors participating in manuscript editing.

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2. Horizontal cell density, morphology, and electrical coupling vary across the mouse retina (In preparation)

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Authors' contributions

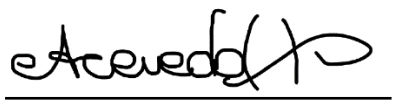
This project was designed by Karin Dedek, Christian Puller, and Ulrike Janssen-Bienhold. Matteo Spinelli contributed to immunohistochemistry experiments and confocal microscopy. Alejandra J. Acevedo Harnecker contributed with intracellular dye/tracer injections, immunohistochemistry, cell reconstruction, and confocal microscopy. Christoph Block and Fabian Schuhmann contributed to the data analysis. Martin Greschner, Ulrike Janssen Bienhold, Karin Dedek, and Christian Puller were in charge of supervision and revision. Figures were prepared by Matteo Spinelli, Alejandra J. Acevedo Harnecker, and Christoph Block and edited by Karin Dedek and Christian Puller. The manuscript is being written by Christian Puller.

14.2 Poster presentation

1. Kick-off and farewell meeting of the DFG research training group molecular basis of sensory biology 1885, online, 23 – 25th March 2021.
2. RTG summer symposium 2022, Berlin, Germany, 5 – 8th July
3. FENS Congress 2022, Paris, France, 9 – 13th July.

15. Versicherung

Hiermit erkläre ich, dass ich diese Dissertation selbständig verfasst und alle benutzten Hilfsmittel vollständig angegeben habe. Des Weiteren lag und liegt diese Dissertation weder in ihrer Gesamtheit noch in Teilen einer anderen wissenschaftlichen Hochschule zur Begutachtung in einem Promotionsverfahren vor. Innerhalb dieser Dissertation wurden die Leitlinien guter wissenschaftlicher Praxis der Carl von Ossietzky Universität Oldenburg befolgt und ich habe im Zusammenhang mit dem Promotionsvorhaben keine kommerziellen Vermittlungs- oder Beratungsdienste in Anspruch genommen.



Alejandra J. Acevedo Harnecker

Oldenburg, 31. January 2024