

Form and Function of S-cone Opponent Circuits in the Primate Retina

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Abstract

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Visual information is carried from the retina by the axons of >20 ganglion cell types, which project throughout the brain. While many of these parallel pathways are cone-opponent, not all the color information leaving the retina is used for color perception, as many ganglion cells instead mediate non-image forming visual functions. However, our understanding of how the visual system uses spectral information is currently limited to the most common cone-opponent ganglion cells and their role in color perception. This thesis aims to expand our understanding of both how and why the visual system extracts color information by investigating the diversity S-cone opponent ganglion cells. I investigated the diverse visual functions mediated by color-coding circuits in the macaque parafovea by combining 3D reconstructions of retinal circuits from serial electron microscopy with single cell electrophysiology, computational models and human psychophysics. When the spatial arrangement of cone-opponent inputs is taken into account, this work reveals cone-opponent receptive fields can contribute to a diverse range of visual functions beyond hue perception, such as edge detection and circadian photoentrainment. More generally, the work in this thesis is inconsistent with the standard view of primate vision that emphasizes cortical processing and limits the role of retinal coding to efficient compression and transmission of visual information. Instead, the complexity and diversity of cone-opponent processing reported here indicates that the retina may play a more active role in the computations defining visual perception and behavior.

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CHAPTER 1

Introduction

“The assumption has always been that the eye mainly senses light, whose local distribution is transmitted to the brain in a kind of copy by a mosaic of impulses. Suppose we held otherwise, that the nervous apparatus in the eye is itself devoted to detecting certain patterns of light and their changes, corresponding to particular relations in the visible world. If this should be the case, the laws found by using small spots of light on the retina may be true and yet, in a sense, be misleading.”

- Lettvin, Maturana, McCulloch & Pitts (1959) What the frog’s eye tells the frog’s brain.
Proceedings of the IRE, 47(11):1940-1951

1.1 Vision

Vision begins in the retina, an outpost of the brain in the back of the eye, where photons of light are absorbed by a two-dimensional array of photoreceptors and converted into an electrical response through a process called phototransduction³⁹⁶. There are two types of photoreceptor, rods and cones. Each operates at different light levels, with rod photoreceptors mediating scotopic vision and cone photoreceptors mediating photopic, or daylight, vision⁷. Most humans are trichromats and have three cone types that are distinguished by the region of the visual spectrum where they are maximally sensitive: long-wavelength (L), medium-wavelength (M) and short-wavelength (S) cones (**Figure 1.1**)^{19,312,265}. While the three cone types are most often considered for their contributions to trichromatic color perception, all daylight vision is the result of some combination of the outputs of the L-, M- and S-cones. Thus, each elementary aspect of vision has some degree of wavelength-dependence, or spectral tuning, from our perception of color, form and motion^{132,131,279} to subconscious non-image-forming visual functions like circadian photoentrainment and visually-

guided reflexes^{136,354,401}. Of these functions, however, the ways in which the three cones combine to give rise to the wide range of colors we perceive are arguably the most intensely studied.

Before delving into the specifics of color vision, it could be useful to first articulate an implicit, internal model for how the visual system operates, i.e. what occurs in the black box between incoming light and visual perception and behavior. Visual processing consists of a series of stages, beginning with a mapping of a specific visual stimulus onto the photoreceptors and ending with the neurons whose activities directly determine behavior or perception³⁵³. At some stages, information may be passed on relatively unchanged, while at others, the information is rearranged or transformed into a new and presumably more useful representation. Wherever the mapping from one stage to the next is not 1:1, a bottleneck occurs where some amount of information is lost and can not be retrieved by subsequent stages. Whether this sort of serial, deterministic approach holds throughout the visual system is debated²⁵⁹, but it appears to be a reasonable approximation of how the early visual system operates.

These abstract ideas apply directly to the neural circuits involved in vision. The first information bottleneck is the cone photoreceptors themselves. The spectral distribution of light varies continuously and trichromacy allows three samples of this distribution. Thus, there is not a 1:1 mapping of wavelength to cones, so information about wavelength will be lost. As a result, any set of spectral distributions that elicit identical activity in the L-, M- and S-cones will be indistinguishable to downstream neurons and at the level of perception. The set of lights that differ in their physical characteristics but are perceptually indistinguishable are called metamers and they were used to determine that humans are trichromatic long before direct measurements of the cones were possible³⁴⁵. This technique of inferring what occurs at a specific stage of visual processing from the information that is lost is a classic approach in vision research that will be revisited in **Chapter Three**.

It follows that the number of cone types places a hard limit on the dimensionality of color vision. However, multiple cone types is only the first requirement for color vision; the neural circuits necessary to extract this information are equally necessary¹⁷³. The poster child for this point is the mantis shrimp, which has twelve photoreceptor types but surprisingly poor wavelength discrimination because downstream circuits do not utilize all possible combinations of the twelve photoreceptors for color discrimination³⁵⁵. Instead, the different photoreceptor types could be

serving different functions, in the same way that our rod photoreceptors are used for night vision rather than tetrachromatic color vision. A goal of this work is to demonstrate that this same concept applies to cone-opponent retinal circuits - some may not be utilized for hue perception and their spectral tuning may instead contribute to other visual functions.

1.2 Color Perception

Our perception of color is an illusion determined by the way our visual systems process light rather than a direct representation of the physical properties of the external world. Put simply, there is no such thing as “red light” or “blue light”. Red and blue are percepts that we often associate with certain wavelengths of light³⁹¹. However, as **Figure 1.2** demonstrates, even that association is an illusion, as the perceived color of an object depends on context, not just the wavelengths of light that are reflected. Like the metamers, these discrepancies between the physical characteristics of light and our perception can be measured and analyzed to reveal the underlying mechanisms of vision.

Trichromatic humans perceive four fundamental hues divided along two opponent axes: red-green and blue-yellow (**Figure 1.4A**)¹⁷⁷. These hues are called fundamental because they are irreducible and every other color can be described as some combination of red, green, blue and yellow³⁶¹. A third opponent channel mediates the fundamental sensations of black and white, which are defined by the absence of any hue sensation. Gray serves as the neutral point between the three opponent axes and is associated with an absence of any sensation. The push-pull relationship between each opponent axis is clear within our own perception. No color is simultaneously red and green or blue and yellow. Staring at a patch of one hue for ~10 seconds, then looking to a white surface will result in an after-image of the opponent hue. Though entirely subjective and arguably lacking in scientific rigor, these phenomena are the result of the neural circuits mediating hue percepts, so they could provide insight into how those circuits work³⁶.

The existence of three broadly-tuned receptors and the phenomena of the three opponent pathways were initially competing theories of color vision supported by Young and Hering^{158,163}. On one side of the debate were the metamer experiments, which carefully measure whether a color can be matched by adjusting the intensities of three primaries, but do not have any clear relationship with the actual appearance of the color. On the other side is the argument that subjective reports of

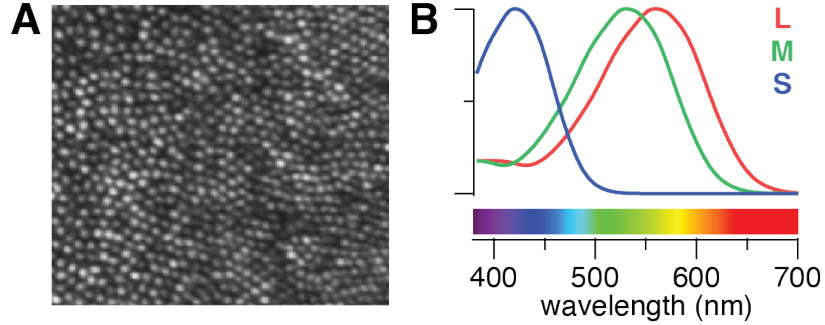


Figure 1.1 (A) An array of the author’s cone mosaic, near the fovea. Image taken with an adaptive-optics scanning light ophthalmoscope by Dr. Xiaoyun Jiang in the lab of Dr. Ramkumar Sabesan. (B) Normalized spectral sensitivities of the L-, M- and S-cone photoreceptors.

color perception, such as the fundamental hues and opponent colors above, may not mean anything at all about the underlying mechanisms of color vision and could just be some side effect of another visual process³⁶¹. Hurvich & Jameson’s opponent process theory reconciled these competing ideas into a single account by developing a method for quantifying color opponency along with a model for how the opponent channels could be obtained by linear transformations of three receptors^{177,170}. Their proposed transformations matched our intuitions about how hue and black-white should be computed - the cone photoreceptors are summed by an achromatic pathway and differenced by two chromatic pathways. The opponent process theory and its influence on subsequent color vision research is discussed further in **Chapter Five**.

1.3 Cone-Opponent Neurons

The first recordings of the color-tuning of neurons in the primate visual system were conducted in the lateral geniculate nucleus (LGN)^{87,384}. Neurons were identified with responses resembling the summing and differencing computations proposed a decade earlier by the opponent process theory. The differencing neurons were called spectrally-opponent because they were inhibited by some wavelengths and excited by others¹⁷⁰. Two main groups of spectrally opponent neuron were identified: “red-green” cells that responded to reds and were inhibited by greens (or vice versa) and a similar set of “blue-yellow” cells that were excited by blues and inhibited by yellows, or vice versa. The summing neurons were spectrally non-opponent and responded either at the onset or the offset of light, regardless of wavelength. A reasonable hypothesis was that the spectrally-opponent neurons that respond to red, green, blue and yellow were in fact responsible for sensations of red,

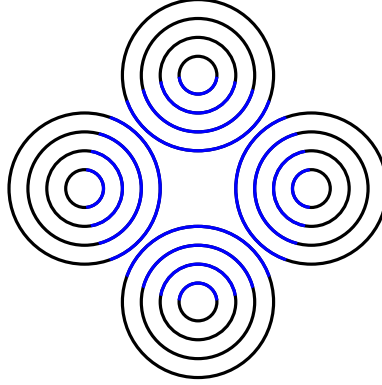


Figure 1.2 An example of a ‘neon color spreading’ illusion where the white background around the blue rings is perceived as light blue. Visual illusions demonstrate both hue and black-white are not fixed properties of the external world. Adapted from Varin (1971) via Bressan (1997)³⁴.

green, blue and yellow, while the spectrally non-opponent neurons mediated sensations of black and white⁹⁰.

At that time, vision research emphasized understanding visual perception at the level of the cortex. Although the diversity and complexity of retinal processing had been demonstrated in invertebrates, the expanded cortex in primates was thought to replace the need for front-loading computations^{25,12}. Instead, eye was thought to act as a camera³¹⁶ with the retina’s purpose to compress visual information down to fit through the optic nerve¹³. This information would be unpacked in the brain, the visual image rebuilt as accurately as possible, and then cortical circuits would perform the computations that define visual perception and behavior (**Figure 1.3**). Within the cortex, each feature of vision - color, motion, form, etc. - was thought to be processed independently and in parallel²³¹. Thus, an unspoken assumption is that color was the only use for the wavelength information carried by spectrally-opponent neurons. Moreover, from this perspective, a neuron responding to color could be safely assumed to play a role in color perception.

The “red-green” cells were later located within the parvocellular layers of the LGN and determined to receive input from ON and OFF midsize retinal ganglion cells (RGCs) comparing L- and M-cones (L vs. M)⁴⁷. The “blue-yellow” cells were located within the koniocellular LGN¹⁶² and many received input from small bistratified RGCs comparing S-cones to L- and M-cones (S-ON/LM-OFF)^{71,46}. The non-spectrally-opponent neurons were located within the magnocellular LGN and their input was parasol RGCs receiving summed L- and M-cone input. Although many of the early surveys reported neurons in the retina⁸¹ and LGN^{350,96,362} with other types of spectral

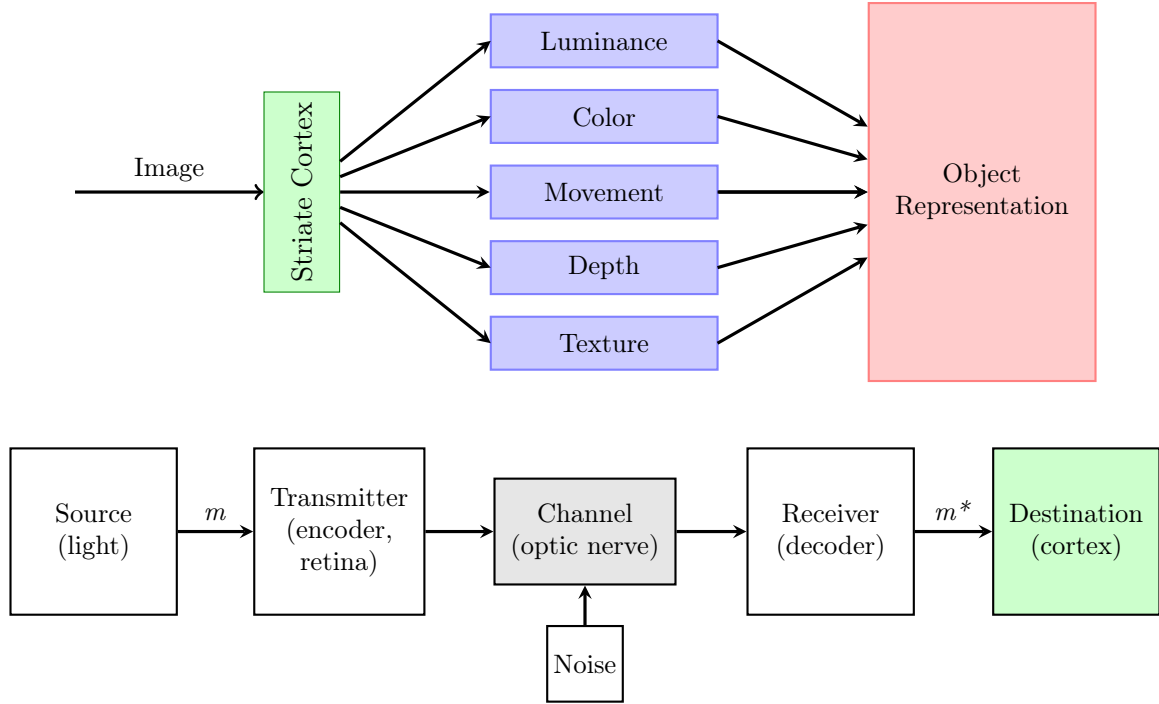


Figure 1.3 (A) A classic model of visual processing emphasizing the role of the cortex. Elementary visual features were proposed to be computed independently and in parallel²³¹. The purpose of the retina (not pictured) was thought to be conveying the image to the cortex as accurately as possible. Adapted from Lennie (1998) *Perception*²²³. **(B)** A model of the early visual system inspired by information theory that proposes how the information from 10^8 photoreceptors can be communicated to the brain through the optic nerve with only 10^6 RGC axons. The message (m) from 10^8 photoreceptors is encoded for optimal transmission through the optic nerve's 10^6 axons by reducing redundancy. Importantly, the encoding is reversible, so the message can be decoded downstream (m^*) and sent to the cortex. When done right, m^* will be as close to m as possible, given the optic nerve's capacity and noise, and one could abstract the entire process and consider the image (as encoded by the cones) impinging directly onto the cortex, as in **A**.

tuning, including S-OFF/LM-ON, L vs. S+M and M vs. L+S, the relative rarity of these neurons prevented systematic study. Thus, the most common cone-opponent neurons, the L vs. M midrange RGCs and the S-ON/LM-OFF small bistratified RGCs, became the cornerstone of modern color vision theories.

1.4 Linking Neurons to Percepts

The discovery of cone-opponent neurons at a time with the opponent process theory was still highly controversial was groundbreaking and the apparent convergence of psychophysics and physiology was very compelling. Although naming the spectrally-opponent neurons "red-green" and "blue-yellow" represented a hypothesized function and not a confirmed one, there were very few skeptics at the time³⁶. In the 60 years since the discovery of cone-opponency in the primate LGN, this confidence in

a direct link between hue perception and the L vs. M and S vs. L+M neurons has been slowly eroded as the underlying circuits were studied in increasing detail. It is now understood that there are no simple connections between the response properties of these neurons and hue perception^{255,336,89,268} and reconciling the discrepancies between the two is a common thread throughout this work. Three unsolved problems at the level of the retina are introduced briefly below and discussed in greater detail throughout the thesis, particularly in **Chapter Two**.

1.4.1 The responses of “red-green” and “blue-yellow” neurons do not match hue perception

The first challenge was posed by the comparison of the cone-opponent neurons to red-green and blue-yellow hue perception. Although there have been varying ideas of the exact cone inputs to the fundamental hue sensations^{177,201}, a convergence of modern evidence from experiments directly measuring hue perception indicates that all three cone types contribute to the two opponent channels: M vs. L+S for red-green and L vs. M+S for blue-yellow^{86,324,331,346,391}. The colors associated with different combinations of cone-opponent activity are shown in **Figure 1.4B**. The hues associated with L vs. M activity are closer to magenta and teal while those associated with S-cone activity are lilac and green. Intuition for the cone inputs to hue perception can also be appreciated from **Figure 1.4B**. For example, blue requires adding a small amount of S-cone input to an M – L neuron while yellow requires subtracting S-cone signals from an L – M neuron (i.e. M+S-L and L-M+S, respectively, or together as L vs. M+S). Thus, the cone inputs to the fundamental hues do not match the most common cone-opponent neurons in the early visual system.

1.4.2 The “red-green” neurons confound spectral and spatial information

A second challenge was posed by analyzing the spatial characteristics of the “red-green” midwidge RGCs, which revealed that they could just as easily serve “black-white” and spatial vision³⁸⁴. This role was originally proposed for the parasol RGCs due to their high contrast sensitivity and lack of spectral opponency¹⁸⁶. However, midwidge RGCs were later determined to set the limit of spatial acuity³¹⁴. As detailed in **Chapter Two**, midwidge RGCs compare cones of different types *and* cones in different locations; thus, they are both spectrally and spatially opponent. As a result, midwidge RGCs respond well to both spatial and spectral contrast and confound the two. From an individual midwidge RGC’s spike output, it is impossible for a downstream neuron to know whether the spikes

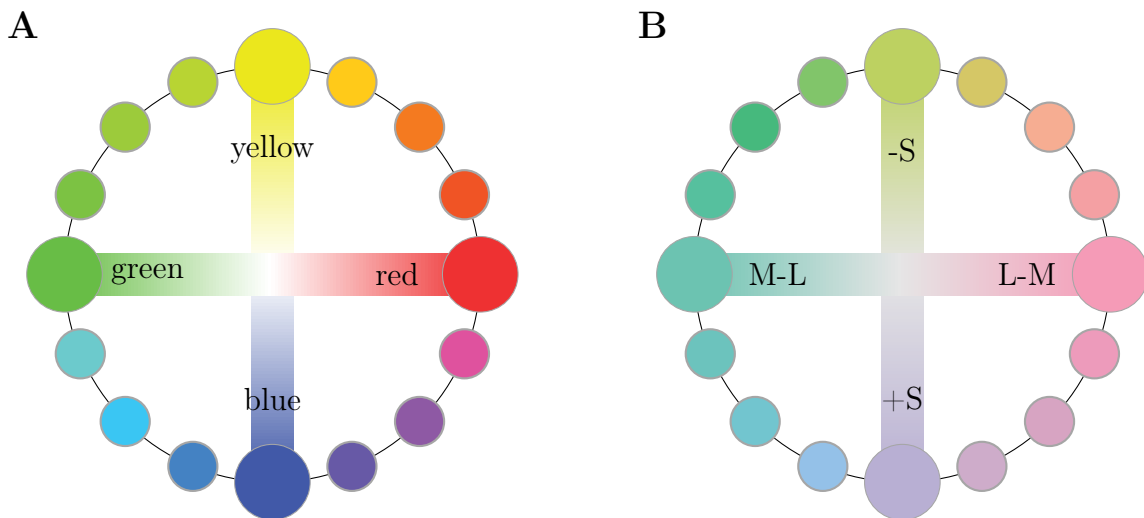


Figure 1.4 (A) The red-green and blue-yellow opponent channels. (B) Colors associated with different cone combinations, plotted in the Derrington-Lennie-Krauskopf (DKL) color space⁹⁶.

were triggered by an edge, color or a colored edge. How the visual system handles this confounded information remains one of the major unanswered questions in vision research.

An implicit assumption in most color vision models is that the spectral and spatial information *must* be separated (or decoded as in **Figure 1.3B**), so that spectral information can contribute to color and spatial information to form through the parallel pathways of **Figure 1.3A**. Exactly how this “de-multiplexing” is accomplished turns out to be far more complex than initially appreciated^{241,187} and the most popular models for doing so fail in reliable ways⁹⁵. Moreover, it is increasingly clear that color vision is never completely separate from other visual features, as depicted in **Figure 1.3**^{379,124,85}. The difficulty of the coding problem posed by the midget RGC’s “poor engineering”²⁴¹ is discussed further in **Chapters Two, Four and Five**.

1.4.3 The numbers of “red-green” and “blue-yellow” neurons do not add up

A third challenge is the strikingly asymmetry in the numbers of L vs. M and S vs. L+M cone-opponent neurons that is not reflected in hue perception. Midget RGCs make up $\sim 90\%$ of the foveal output while small bistratified RGCs make up just 3% ^{68,67}. Moreover, the S-OFF neurons initially proposed to mediate yellow proved to be elusive in the retina^{236,202,80}. Early surveys of cone opponency in the retina did report blue/S-OFF neurons, but at a much lower frequency than the S-ON small bistratified RGCs⁸³ and, as a result, they received very little research attention, were

largely overlooked by the most influential color vision models⁸⁹ and eventually their existence was denied entirely^{347,221}. It took another fifty years before these rare S-OFF neurons were identified as S-OFF midget RGCs and intrinsically photosensitive retinal ganglion cells (ipRGCs)^{189,73}. As is tradition for cone-opponent RGCs, both have been proposed to mediate hue perception, specifically the perception of red¹⁸⁹ or yellow hues^{70,339}. However, neither is a good fit for yellow hue perception as the ipRGCs lack the necessary spatial resolution²⁶⁰ while the S-OFF midget RGCs lack the S-cone selectivity necessary to reliably signal LM-S outside the central retina (discussed further in **Chapter Four**). Moreover, when compared to the near symmetry between ON and OFF midget RGCs¹⁹⁵, neither ipRGCs nor S-OFF midget RGCs make a compelling OFF counterpart to the small bistratified RGC.

1.4.4 The ‘Standard Model’

To summarize, there are significant discrepancies between hue perception and the response properties of the most common cone-opponent neurons in the early visual system³³⁶. Ultimately, these discrepancies were fit to the model of the early visual system shown in **Figure 1.3B** and even deemed optimal in certain regards. If retinal circuits are compressing information for optimal transmission to the brain, rather than actively contributing to perception in any specialized way, then a lack of correspondence with visual experience is not an issue. **Chapter Two** discusses the currently-favored hypotheses for how the cone-opponent RGCs could be decoded and recombined to mediate hue and spatial vision. These corticocentric models lack direct evidence, are based in outdated research and have struggled to accommodate new findings, yet they remain popular as there is a general feeling that the cortex *should* be able to figure it out. The work in this thesis will ask whether the solution may not be increasingly advanced cortical computations but rather an investigation of the untapped sophistication of retinal circuits^{311,44,324}.

1.5 The Retina

As the most accessible part of the central nervous system, the retina is famously known as “an approachable part of the brain”¹⁰⁴. The retina contains five main classes of neuron: photoreceptors, horizontal cells, bipolar cells, amacrine cells and RGCs. These five classes and their defining features are roughly conserved in all vertebrate retinas^{182,12}. The following sections addressing each type of

retinal neuron are meant to provide a brief overview of the retina while also making a point. The complexity and diversity of visual processing vastly exceeds the functions traditionally attributed to the retina^{135,364}.

1.5.1 Photoreceptors

As shown in **Figure 1.1B**, each cone type has a different spectral sensitivity, which defines the probability that a photon of a given wavelength will be absorbed. The S-cone’s spectral sensitivity means photons of 600 nm are less likely to be absorbed than 420 nm, but once the photon is absorbed, the same phototransduction cascade occurs, regardless of the photon’s wavelength. In other words, the cones are colorblind because their output encodes just one variable (photon absorption). This ‘principle of univariance’ and its consequences for color vision are discussed further in **Chapter Two**.

Unlike most neurons, cones are depolarized and releasing glutamate in the absence of any stimulation (i.e. in darkness). When light is absorbed, the cone hyperpolarizes and reduces glutamate release. The glutamate is released onto an impressively complex and highly organized system of bipolar and horizontal cell processes^{57,153}.

1.5.2 Horizontal cells

In the outer retina, two types of horizontal cell provide antagonistic feedback to cones and feed-forward input to bipolar cells^{356,296}. HI horizontal cells contact only L- and M-cones while HII horizontal cells extensively innervate S-cones and make sparse contacts with the outer edges of L- and M-cone pedicles⁷². As explained in **Chapter Two**, horizontal cell feedback establishes both spatial and spectral opponency at the level of each individual cone’s output by allowing comparisons of cones in different locations and of different types, respectively^{366,278}. In fact, the response properties set by horizontal cell feedback appear relatively unchanged from the cone outputs to the LGN, at least when tested with classic linear stimuli like drifting gratings, spots and annuli.

Despite the clear understanding of how horizontal cell feedback shapes RGC responses, many open questions remain, including the synaptic mechanisms responsible for their feedback^{185,199}. Moreover, several other aspects of horizontal cell function are virtually unstudied, particularly in the primate, such as their feed-forward input to bipolar cells²⁹⁶ and the role of HI and HII axon

terminals, which contact rods and cones, respectively, and act as independent dendritic subsystems^{230,271}.

1.5.3 *Bipolar cells*

The ~12 types of bipolar cells decompose the cone photoreceptors' univariant output into 12 parallel channels differing in their polarity, spatial, temporal and spectral properties^{116,359}. The most notable of these transformations is the division into ON and OFF bipolar cells, through the expression of mGluR6 and AMPA/kainate receptors, respectively, which have opposite responses to glutamate^{365,154}. The distinction between AMPA and kainate receptors on OFF bipolar cells along with the stereotyped distance each is located from the cone's glutamate release reveals another set of parallel pathways distinguished by sustained and transient responses, respectively⁹⁷. The number and types of cones each bipolar cell contacts establish additional parallel pathways, differing in their spatial and spectral properties^{358,359}.

Within the inner retina, the bipolar cells axon terminals provide excitatory glutamatergic output to amacrine and RGCs in a highly organized way. Each type stratifies at a distinct depth within the inner retina creating many parallel vertically-stacked representations of the original information present in the cone outputs³¹³. By convention, the inner retina is typically divided into five sublamina with S1 located closest to the bipolar cell somas and S5 adjacent to the RGC somas. However, the degree of stratification is far more complex than just five divisions. The inner retina can also be divided by function with OFF cells stratifying in S1-S2 and ON cells stratifying in S3-S5²⁷¹. In addition, the axon terminals of some ON bipolar cells, such as the S-ON and DB6 bipolar cells, make a few synapses in S1, forming an "accessory ON layer"^{229,46,169}. Further divisions can be made by kinetics, as bipolar cells with transient responses and spike generation are found in S2-3 while bipolar cells with sustained responses are found towards the edges of the inner retina^{301,300,116}.

1.5.4 *Amacrine cells*

Amacrine cells are the most diverse and least understood class of retinal neuron. Describing the defining features of amacrine cells is a difficult task because they exhibit considerable diversity along almost every possible dimension. Broadly, amacrine cells modulate the visual information passing through the inner retina, receiving bipolar and/or amacrine cell input and providing output

to bipolar cells, other amacrine cells and RGCs. Amacrine cell somas are found along both edges of the inner retina and their dendrites stratify within the inner retina diffusely or in 1-3 distinct tiers. Most amacrine cells are axonless, although a subset called polyaxonal amacrine cells have multiple axons which extend laterally through the inner retina and occasionally to the outer retina⁶⁶. The output synapses of amacrine cells use inhibitory neurotransmitters GABA or glycine³⁷⁷, though there are a few notable exceptions such as the glutamatergic amacrine cells¹⁵⁵. Also, the net effect of amacrine cell output onto a post-synaptic neuron is not always inhibitory as there are situations where amacrine cells act by releasing inhibition, thus producing a net excitatory effect²³⁸. Several types of amacrine cell also release neuromodulators such as acetylcholine, dopamine or nitric oxide^{66,174}.

Of the 40+ amacrine cell types in the mammalian retina³⁹⁵, very few have been placed in the context of specific circuits or visual functions, particularly in primate. However, the distribution of inputs and outputs along an amacrine cell's dendritic field can provide some insight into the diverse computations they perform, which are often thought of as variations on a small number of themes or motifs^{377,141}. For example, inputs and outputs could be tightly clustered in varicosities where they perform independent local computations¹⁴³, they could be distributed along a dendrite to provide selectivity for motion in a specific direction¹¹⁸, or separated into different strata to carry input from one strata to another²³⁹. The polyaxonal amacrine cells provide another example, collecting inputs along a relatively small dendritic field, then transmitting that information along their axons over long distances (up to several millimeters) using action potentials^{78,140}. Amacrine cells are investigated in **Chapter Six** and **Seven**, and in work published outside this thesis²⁸¹.

1.5.5 *Ganglion cells*

RGCs are the retina's only output neuron. Their axons make up the optic nerve and convey visual information to the brain in binary, as all-or-nothing action potentials. Each of the 17+ RGC types forms a mosaic that tiles the retina and is thought to extract a distinct representation of the visual world²⁴⁹. Thus, each location in the visual field is represented 17+ times in the retinal output. The details of each representation are determined by the bipolar and amacrine cell inputs RGC dendrites receive within the inner retina. Each RGC type stratifies in one or two areas of the inner retina and many are split into ON and OFF subtypes. Given the functional stratification of the

inner retina, it is not surprising that the ON and OFF parasol RGCs have ON and OFF responses, respectively, while the broad thorny RGCs have both ON and OFF responses²⁹⁷.

The axons of RGCs project throughout the brain and serve both image-forming and non-image-forming vision. A brief and incomplete list of retinorecipient regions includes the lateral geniculate nucleus, superior colliculus³⁷⁵, pulvinar²⁰⁷, accessory optic system³⁵¹, suprachiasmatic nucleus and the olivary pretectal nucleus¹⁴⁹. However, the organization of RGC inputs to the different regions of the LGN is by far the most studied and best understood, particularly the projections of the midget, parasol and small bistratified RGCs to the parvocellular, magnocellular and koniocellular layers of the LGN. The details of midget, small bistratified and parasol RGC circuitry are discussed in **Chapter Two**, **Chapters Four** and **Six**, and in my work published outside this thesis^{237,281,134}, respectively. It is worth noting that these three pathways have complexities that are difficult to fit into any simple, textbook model²⁵⁰. Each layer receives input from multiple RGC types, many that have only been characterized anatomically^{289,375}. For example, the koniocellular pathway is often described as mediating blue-yellow color vision, many of the koniocellular LGN neurons and their RGC inputs have complex receptive fields without S-cone input^{289,110}.

1.5.6 Ganglion cell photoreceptors

The discovery of ipRGCs²² is one of the biggest discoveries in vision and retinal circuitry in the past few decades and they continue to challenge long-standing ideas about the vision^{277,169,294,337}. Rods and cones were long thought to be the only retinal neurons capable of phototransduction. However, ipRGCs also express a photopigment called melanopsin and can respond directly to light. The spectral sensitivity of melanopsin peaks at 483 nm and is ~1000x less sensitive than the cone opsins. However, ipRGCs are otherwise traditional RGCs in the sense that they receive input from traditional rod and cone pathways. As mentioned in **Section 1.4.3**, the cone inputs to primate ipRGCs confer a rare LM-ON/S-OFF cone-opponent response⁷³.

The ipRGCs are highly-conserved and the two types with the strongest melanopsin expression (M1 and M2 subtypes) are found in almost all mammalian species¹⁰¹. There are at least three physiological and anatomical types of ipRGC in primate, however, the links between the two remain to be fully confirmed^{261,52} as only one study thus far has combined anatomy and physiology⁷³. In primates, ipRGCs project to the suprachiasmatic nucleus, the olivary pretectal nucleus, the

superior colliculus, pregeniculate complex and the LGN¹⁴⁹. The role of ipRGCs in the circadian photoentrainment and the pupillary light reflex are well-established in mouse and supported by work in humans^{130,341}. There is also considerable interest in whether and how the projections to the LGN influence perception³. The different subtypes and their potential roles in vision are investigated in **Chapters Six** and **Seven**.

1.5.7 Fovea

In addition to the diversity of distinct cell types, there is considerable within-type variation as a function of retinal location^{27,370,165}. The most notable regional variations in the primate are the changes with retinal eccentricity that occur between the fovea and peripheral retina. The fovea is a small, highly specialized region of the retina that mediates most visual perception³⁷. Foveal vision is vastly different from that in the retinal periphery and is best known for superior color and spatial acuity. This specialized role in vision is clearly reflected by the retina, beginning with a high concentration of cones, exclusion of rods and the displacement of other retinal neurons away from the light path towards the cone outer segments¹⁶¹. Foveal neurons are densely-packed and have smaller dendritic fields that receive input from a smaller number of upstream neurons, relative to the periphery.

Midget RGCs are the hallmark of the primate fovea and make up ~90% of the foveal output⁶⁸. Their circuitry within the fovea is reviewed in **Chapter Two**. There is clear evidence that physiology varies with eccentricity - foveal cones are slower and foveal midget RGCs receive less inhibition within the inner retina³³². However, understanding of the physiology of foveal neurons lag behind anatomical and psychophysical work due to the technical challenges of recording from the primate fovea. Accordingly, it is worth noting that our understanding of the response properties of primate RGCs, including those proposed to mediate color, relies heavily on recordings from the periphery, where color vision is quite poor.

The work here strikes a balance between technical difficulty and applicability to color perception by focusing on the central retina, the area immediately outside the fovea. That retinal anatomy and physiology changes so dramatically with retinal eccentricity is a sign that the retina is specialized for specific visual functions and can be understood best in the context of those functions¹². The structure of the fovea, the lack of rods in the foveal center and the sacrificed acuity in the

periphery all demonstrate that the retina does far more than agnostically maximizing information transmission.

1.6 S-cones

The three cone types can be considered as two functional groups: S-cones and L/M-cones. The split between L- and M-cones occurred ~ 30 million years ago (“recently” on an evolutionary time-scale). Accordingly, the DNA sequences of the genes for L- and M-opsins (*OPN1LW* and *OPN1MW*, respectively) are 98% identical and lie in tandem on the X-chromosome²⁶⁹. The S-opsin gene (*OPN1SW*) is far more distinct and is located on chromosome 7²⁶⁵. As shown in **Figure 1.1B**, the spectral sensitivities of the L- and M-cones overlap substantially while the spectral sensitivity of the S-cones is further removed.

While there are subtle morphological differences between S-cones and L/M-cones¹⁹³, the L- and M-cones cannot be distinguished from each other anatomically. S-cones are electrically isolated while L/M-cones are gap junction coupled to each other, which allows signals to spread between neighboring L/M-cones, blurring the distinction between their already overlapping spectral sensitivities¹⁶⁸. While the ratio of L- and M-cones varies considerably between individuals, S-cones make up a reliable 5-10% of all photoreceptors^{65,50}. There is some variation in the density of S-cones with retinal location: S-cones are absent from the very center of the fovea³⁸⁵, their density peaks immediately outside the fovea and more are found in nasal retina than in the temporal retina⁶⁵. S-cones are noisier than L/M-cones and have slower responses that change less with background light intensity¹⁸.

The sparsity of S-cones and the defocus of short-wavelength light due to chromatic aberration reduces the resolution of S-cone exclusive circuits²⁵¹ and is seen as evidence that they evolved to provide low-resolution color vision³³⁶. S-cone responses are often assumed to indicate a role in color perception, however, the number of areas receiving S-cone input has grown to include those not likely to be involved in color perception⁶⁰. A central theme to this thesis is that the distinction between S-cone circuits for hue and for functions other than hue begins in the retina.

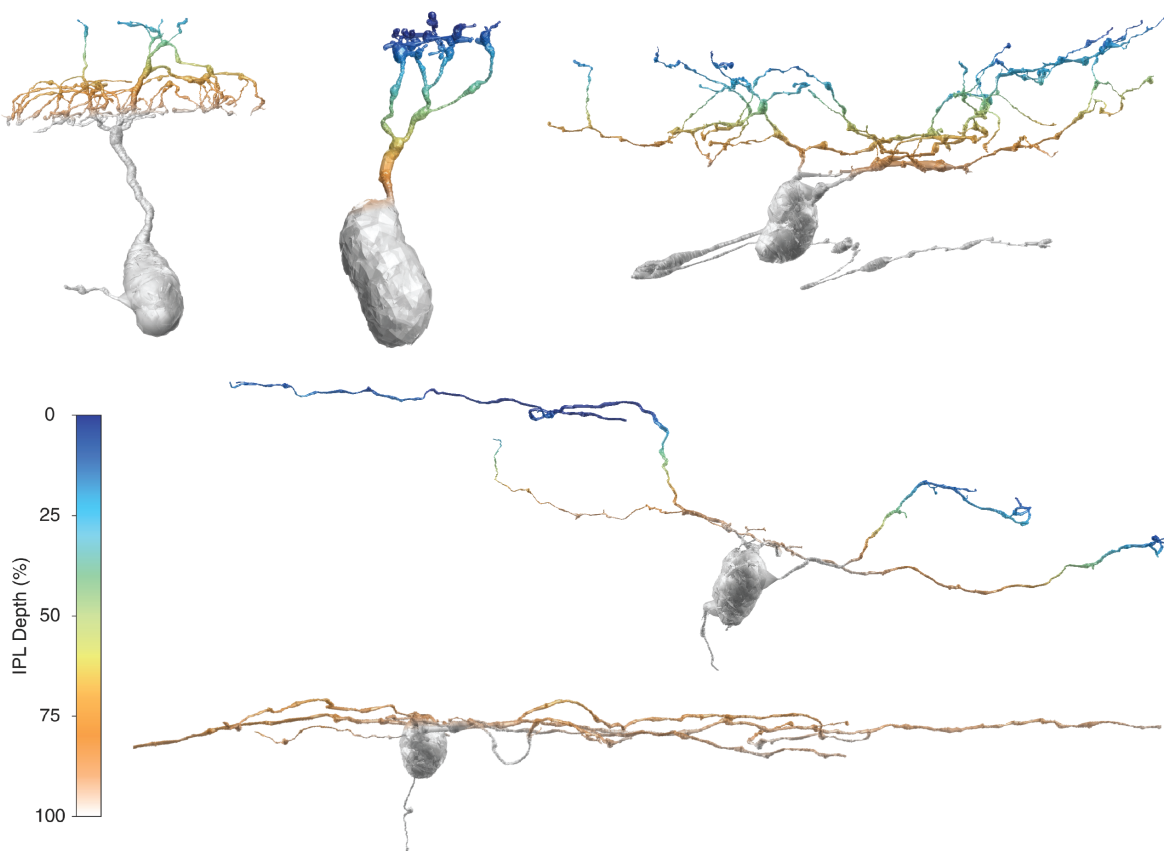


Figure 1.5 3D reconstructions of five primate RGC types carrying S vs. L+M information. Dendrites are colored by their stratification in the inner retina (S1 begins at 0% and S5 ends at 100%, as in the scale bar to the bottom left). The top three from left to right: small bistratified RGC, S-OFF midget RGC, large bistratified RGC. The middle and bottom rows show M1 and M2 ipRGCs, respectively. Note: reconstructions are not sized to the same scale.

1.6.1 *S-cone Circuits*

As discussed in **Chapter Two**, there is little evidence that downstream circuits distinguish between L- and M-cones. In contrast, S-cones have dedicated circuits that form a sparse subsystem within the larger and more complex retina. The best-studied, and arguably the only well-studied S-cone circuit begins in the outer retina with a dedicated S-cone ON bipolar cell, which receives input from 1-3 S-cones^{240,198}. HII horizontal cells, which predominantly contact S-cones^{51,72} and also provide feed-forward input to S-ON bipolar cells²⁹⁶. The S-ON bipolar cell provides excitatory input to the small bistratified RGC⁴⁶, which carries S-cone signals to the “blue-ON” cells of the koniocellular LGN²⁴⁸. This canonical S-cone circuit was long thought to be the only retinal output for S-cone signals and a dedicated pathway for “blue-yellow” color vision²⁴⁶.

There is no S-cone exclusive OFF bipolar cell similar to the S-ON bipolar cell, and, until recently, S-cones were thought to lack an excitatory OFF pathway entirely, similar to the rod circuit²⁸. As a testament to the strong bias towards only the most common retinal neurons, OFF midget bipolar cell contacts at S-cones were only first reported in 2003¹⁸⁹ and have remained highly controversial^{221,347,352} (see **Chapter Four**).

OFF diffuse bipolar cells also contact S-cones to varying degrees, although L/M-cones likely dominate their responses as the fraction of S-cone input is generally small²²⁰. Though a functional role has not been identified for S-cone input to diffuse OFF bipolar cells¹²¹, the stereotyped preferences of each subtype for S-cone input suggests some unknown role may exist. DB2 and DB3b bipolar cells make up around 25% and 15% of the S-cones OFF bipolar cell output while DB1 and DB3a bipolar cells receive just 3% and 2%, respectively³⁵⁸. S-cones have negligible contact with ON diffuse bipolar cells²¹⁹ as the S-ON bipolar cells makes up $\sim 97\%$ of the S-cone's output to ON bipolar cells³⁵⁹. The giant ON bipolar cell contacts a sparse subset of cones and was proposed to be S-cone selective¹⁸⁰, however, subsequent work revealed they contact all photoreceptor types, including rods³⁵⁹.

Amacrine cells receive about half of the S-ON bipolar cell's output⁴⁶, yet very little is known of their identity. S-cone-specific amacrine cells receiving only S-ON bipolar cell input have been reported in the mammalian retina and are proposed to be present in primates⁵⁴ (see **Chapter Six**). In addition, the AII amacrine cell forms gap junctions with S-ON bipolar cells to transmit signals from rod photoreceptors at low light levels¹²².

Although the idea of the small bistratified RGC as the sole retinal output for S-cone signals remains the textbook view, the number of S-cone RGCs has been increasing. As mentioned in **Section 1.4.3**, there are two S-OFF RGCs: the S-OFF midget RGCs and the ipRGCs. Like all OFF midget RGCs, the S-OFF midget RGC receives input from OFF midget bipolar cells, a subset of which contact S-cones. Input from an S-cone-contacting OFF midget bipolar cell will confer an S-OFF/LM-ON spectral tuning in the central retina, where each midget RGC receives input from a single midget bipolar cell. However, this spectral signal will be diluted outside the central-most retina where OFF midget RGCs also receive input from L/M-OFF midget bipolar cells^{390,121}. The circuit responsible for the S-OFF response in ipRGCs is identified in **Chapter Six**. There are also preliminary reports of S-ON responses in large bistratified RGCs and S-OFF responses in large

sparse RGCs, but these have not been studied systematically^{74,69}.

In total, at least five distinct RGC types carry S vs. L+M information (**Figure 1.5**). Further, it is striking that a single spectral signal (S-OFF/LM-ON) is carried by both the evolutionarily conserved ipRGCs mediating non-image-forming functions and the phylogenetically recent midget RGCs responsible for color and high visual acuity. This diversity of neurons carrying a spectral signal that does not correspond with hue perception indicates that we do not fully understand how the visual system uses wavelength information.

1.7 Evolution

Nothing evolves *de novo* and every aspect of the primate retina arose as some modification of an existing ancestral retina²⁶⁷. Accordingly, evolution provides a useful context for understanding the potential roles of specific retinal circuits in vision^{268,257}. While we think of our conscious experience of color as a uniquely primate capacity, the basic neural substrate for our color perception, cone-opponent neurons, are found in most species, including other mammals,^{348,330,160} fish⁴⁰², turtles⁴ and even fruit flies^{328,157}. Thus, these species may not have the perceptual experience of color, but they do have the capacity for color vision, which is formally defined as the ability to distinguish lights based on wavelength independent of intensity¹⁷³. Research on the evolution of primate color vision typically emphasizes what distinguishes primates by focusing on the advantage conferred by trichromacy^{103,276}. This thesis will take the opposite perspective and focus on the aspects of mammalian vision that were worth conserving. Two questions are asked. What specific visual functions are mediated by the cone-opponent circuits in other species and are any of these functions conserved? More broadly, can an understanding of how other species use wavelength information apply to the primate retina?

The emphasis here will be research in the mammalian retina, which has a form of dichromacy similar to our own trichromacy, though it is worth noting that other species, such as the zebrafish, can also provide compelling insight³⁹⁸. Most mammals have a sparse mosaic of short-wavelength cones²⁶² along with a short-wavelength sensitive ON bipolar cell homologous to the S-ON bipolar cell^{240,156,295}. This has been called the “primordial color vision circuit” and is proposed to be the precursor for our own blue-yellow color perception circuitry²⁵⁴. Despite this shared circuit, it has long been thought that there was little to learn about primate color vision from non-human

animal models, particularly the mouse. There is good reason for thinking this way, given that our color perception involves features thought to be unique to primates, such as trichromacy, the fovea, midget RGCs, the parvocellular LGN and the visual cortex. Our expanded cortex has particularly influenced primate vision research, leading to the widely-held view that the retinal circuits found in other species were replaced through evolution by downstream cortical processing (a.k.a. the smarter the brain, the dumber the retina)¹².

What could we hope to learn from an animal like the mouse that has low visual acuity and a gradient of opsin co-expression rather than three distinct cone types⁸? The obvious asset of the mouse retina is the opportunity to obtain a clear answer for the role of a specific neuron in vision. Primate research cannot compete with the ability afforded by transgenics to label, target and perform causal manipulations individual classes retinal neurons. Without this capability, we are left correlating the response properties with percepts and psychophysical functions. This approach is fraught with difficulty, prone to misinterpretations and after sixty years of carefully studying the midget, parasol and small bistratified RGCs, each is mired in controversy. It is worth finding common ground with a species that has more success linking retinal circuits to downstream functions.

1.8 Feature Detection

The detailed characterization of mouse RGCs over the past two decades has revealed several insights on retinal function that could apply to primate. The first insight is the sheer functional diversity of the retinal output - over 40 RGC types can be distinguished functionally by their distinct temporal, spatial and spectral tunings¹¹. A second insight is that the mouse RGCs process information similar to the striate cortex in **Figure 1.3A**. Many selectively compute specific visual features like direction¹⁷, object motion¹⁰ or uniformity¹⁷⁵ and respond with a univariant spike output that can be read out directly without a downstream decoding step¹³⁵. This should make anyone studying primate RGCs with classic methods uncomfortable, as many of the specific features each RGC encodes would have been difficult to guess from independent measurements of their spatial, spectral and temporal tuning.

Although the idea of RGCs as feature detectors has been growing in popularity recently, it is a well-established idea first put forward in the classic paper “What the Frog’s Eye Tells the Frog’s Brain”²²⁷. Lettvin & colleagues (1959) identify a set of highly specific RGCs, such as neurons that

respond to small moving objects and could serve as “bug detectors”. They conclude that “the eye speaks to the brain in a language already highly organized and interpreted, instead of transmitting some more or less accurate copy of the distribution of light on the receptors.” The authors were very clear that this conclusion applied to just the frog, which “does not seem to see”, at least in the way that humans do. While true in some ways, this observation alone isn’t sufficient to justify using different sets of assumptions for primate and non-primate research. Though evolution is not often considered in terms of computational strategies, it is increasingly clear that the computations performed in other retinas were not abandoned during evolution to primate^{206,237}. It could be worthwhile to consider that the general principles of retinal computation in a frog or mouse eye may have more in common with those in the human’s eye than previously thought. Or perhaps more accurately, that the retina has more in common with the classic view of the primary visual cortex (V1). The feature detection above bears a striking resemblance to the description of **Figure 1.3A** by Lennie (1998): “One might think of V1 as transmitting *decisions* (albeit about elementary attributes of the image) to higher cortical areas, but perhaps little of the information that gives rise to these decisions.”²²³

How is this relevant for cone-opponent neurons in the primate retina? To summarize, the limitations of the model for primate vision discussed in **Section 1.4** and in **Figure 1.3** are increasingly accepted, along with the idea that the retina is far more than a passive conduit for visual information²⁵⁰. However, classic color vision models established from this perspective remain and must be updated. RGCs as feature detectors provides a framework for understanding why we have five distinct RGC types carrying a spectral signal that does not match hue perception. Rather than being extracted out to contribute to color perception, the spectral opponency may operate in concert with the RGC’s other receptive field properties to encode a specific visual feature. In other words, it could be useful to stop thinking of spectral opponency independently and instead consider it in the context of the RGC’s other response properties. The question, then, is what feature does each S vs. L+M neuron encode?

1.9 Outline

This thesis aims to look beyond the role of spectrally-opponent RGCs in hue perception and explore the possibility that wavelength information could be used for other purposes. In particular, this

work will investigate whether spectrally-opponent RGCs serve as feature detectors. This perspective on RGC function is established in other species but runs counter to many long-held intuitions about primate vision and thus meets considerably resistance. As such, an important goal of this work is to establish this approach as a valid line of inquiry capable of providing compelling answers to some of the difficult questions that could not be addressed by existing models.

Chapter Two continues this discussion through a review on the co-evolution of color vision models and our understanding of the receptive field structure of cone-opponent RGCs. **Chapter Three** investigates the dimensionality of color perception downstream of the cone photoreceptors to determine whether the neural circuits for hue perception take full advantage of trichromacy. **Chapters Four** and **Five** explore the idea that L vs. M and S-OFF midget RGCs are not “multiplexing” or even confounding spectral and spatial information, but are instead edge detectors capable of responding to all edges, including those defined by spectral contrast. **Chapters Six** and **Seven** detail the S-cone opponent circuits for ipRGCs and explore how wavelength information could be used for non-image-forming vision.

CHAPTER 2

Reconciling Color Vision Models with Midget Ganglion Cell Receptive Fields

The study presented in this chapter has been published as:

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2.1 Summary

Midget retinal ganglion cells (RGCs) make up the majority of foveal RGCs in the primate retina. The receptive fields of midget RGCs exhibit both spectral and spatial opponency and are implicated in both color and achromatic form vision, yet the exact mechanisms linking their responses to visual perception remain unclear. Efforts to develop color vision models that accurately predict all the features of human color and form vision based on midget RGCs provide a case study connecting experimental and theoretical neuroscience, drawing on diverse research areas such as anatomy, physiology, psychophysics, and computer vision. Recent technological advances have allowed researchers to test some predictions of color vision models in new and precise ways, producing results that challenge traditional views. Here, we review the progress in developing models of color-coding receptive fields that are consistent with human psychophysics, the biology of the primate visual system and the response properties of midget RGCs.

2.2 Introduction

The first stage of visual processing occurs in the retina, an outpost of the brain located at the back of the eye. Under photopic conditions, photons of light are absorbed by three types of cone photoreceptor (**Figure 2.1A**), processed by five main classes of retinal neuron, then visual signals are conveyed to the brain by the axons of retinal ganglion cells (RGCs)³⁷¹. Midget RGCs make up a large majority of all RGCs in the central retina, where each L- and M-cone provides the sole direct input to an ON and OFF midget RGC circuit (**Figure 2.1C**)^{373,374,195}.

The midget RGC receptive field has a center-surround organization²⁰⁵. In the central retina, this receptive field compares the photon catch in the single L- or M-cone center to the photon catch in neighboring L/M-cones in the surround (**Figure 2.1C**). Since this configuration compares the activity of cones that differ in both spatial location and spectral sensitivity, midget RGCs have been implicated in both color and spatial vision^{320,245}. Mammalian RGCs have been described as acting as feature detectors, with different types showing specificity for motion, form or color conferred by the spatial, spectral, and temporal characteristics of their receptive field^{123,135,11}. Here, we review evidence for the role of midget RGC receptive fields as the first step for detection of two elementary visual features, (1) hue detectors which encode information about spectral reflectances of surfaces as red, green, blue and yellow percepts, (2) high acuity edge detectors which encode the boundaries of objects as required for form vision.

Because their receptive fields exhibit both spectral and spatial opponency, midget RGCs respond to both chromatic and achromatic edges and thus confound the two³⁸⁴. Like all RGCs, midget RGCs encode and transmit information to the brain in binary, as all-or-nothing action potentials. A downstream neuron has no way of knowing, from an individual midget RGC's response, whether the midget RGC responses represent the chromatic or spatial structure of a stimulus. At the level of perception, however, we can distinguish between achromatic and equiluminant chromatic edges, even though individual midget RGCs cannot. How and where the spectral and spatial information encoded by midget RGCs is extracted remains one of the most important unanswered questions of primate vision.

Midget RGCs provide, arguably, the best model for linking low-level receptive fields to perception. Understanding how color and spatial information are encoded may provide insight into general

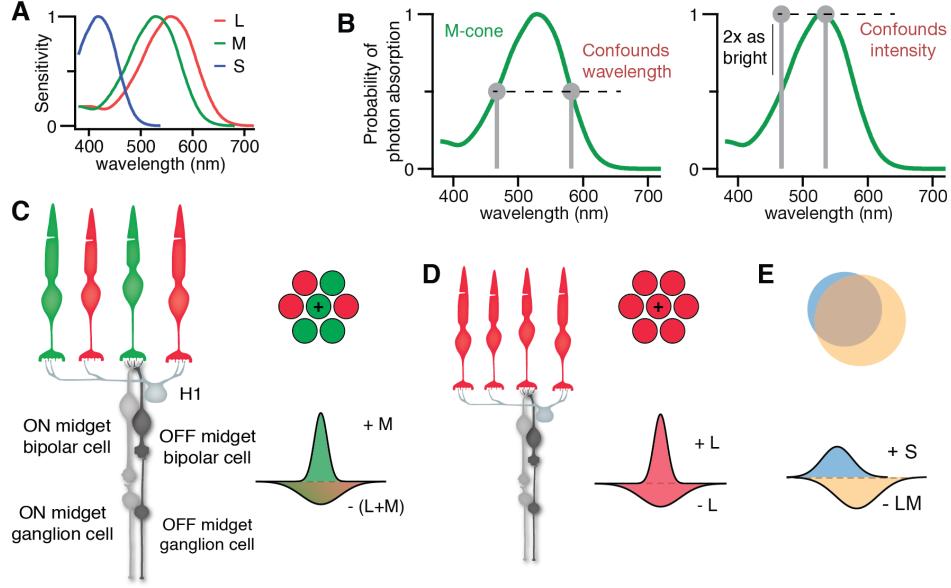


Figure 2.1 Color-coding receptive fields of the primate retina. (A) The normalized spectral sensitivities of human L-, M-, and S-cone photoreceptors. (B) Demonstration of monochromatic lights that elicit the same probability of photon absorption, and thus elicit the same response from M-cones. On left, 467 and 582 nm lights are indistinguishable, despite a 115 nm difference. On the right, a 467 and 535 nm lights of different intensities can be confounded by adjusting the intensity of the 467 nm light. (C) Circuit diagram of the upstream input to ON and OFF midget RGCs. (D) Same as in (C), but for the midget RGCs of a dichromat. (E) Small bistratified RGC receptive field diagram illustrating the lack of perfectly coincident spectrally opponent receptive fields as required for pure color cells.

organizational principles employed by neural circuits to parse specific features of a stimulus. Furthermore, restoration of color and spatial vision are an important goal for retinal prosthetics, some of which must replace the upstream circuitry that defines the midget RGC receptive field³⁹⁹. Efforts to restore these fundamental aspects of visual perception may benefit from a better understanding of how they are computed in normal vision.

2.3 Receptive Fields

All receptive fields are built from the photoreceptor outputs (**Figure 2.1A**). The photoreceptors' output encodes a single variable: the number of photons absorbed^{316,19}. An important implication is that wavelength and intensity are interchangeable and, under the right conditions, any two lights differing in wavelength can be "substituted silently" for each other¹¹⁴. For example, the probability of photon absorption by an M-cone is the same for 467 and 582 nm lights, thus the response of the M-cone shown in **Figure 2.1B** to the two lights will be indistinguishable. Meanwhile, a 535 nm light with twice the probability of photon absorption can be matched by doubling the intensity of

the 467 nm light.

The visual system extracts information about wavelength and spatial contrast by virtue of receptive fields that compare the outputs of multiple cones. The basic computation for extracting wavelength is a comparison between cones of different spectral types, while spatial contrast requires comparing neighboring cones at different spatial locations, regardless of type⁴⁴. The characteristics of receptive fields form the foundation of each color vision model discussed here.

2.3.1 What is the Optimal Receptive Field for Spatial Vision?

Because midget RGCs are implicated in high acuity form vision, any discussion of their color-coding role must also include their role in spatial-coding. The first step of spatial vision requires delineating the boundaries of objects, essentially performing an edge detection task.

Spatial Opponency. By comparing the relative activity of cones at different locations, spatially opponent receptive fields signal spatial contrast rather than raw quantal catch³⁴². For low-level edge detectors, circularly symmetric center and surround receptive fields are optimal and will provide sensitivity to all edges, regardless of their orientation²⁴¹.

Spectral Opponency. While spatial vision is sometimes assumed to operate only on light intensity^{241,24}, equiluminant edges are also common in natural scenes¹⁵¹. Accordingly, an optimal edge detector would be sensitive to all edges regardless of whether the edge is defined by a change in wavelength or intensity. Thus, an optimal edge-detecting receptive field might not just be spatially opponent, but also spectrally opponent. In this case, the purpose of spectral opponency is not to signal the hue of a surface but rather an edge defined by spectral contrast.

2.3.2 What is the Optimal Color-Coding Receptive Field for Hue Perception?

In the natural world, most colors we perceive are from lights reflected from objects. The purpose of hue perception is to provide information about the surface reflectance of objects, which, in turn, tells us about their internal contents or state. For example, we know the ripeness of fruit and when children are getting sunburned from their surface colors. However, there are significant challenges to this task. Individual cones themselves are not selective for the distribution of wavelengths reflected

from a surface. If L-cones are active, light could be coming from a red surface reflecting only long wavelengths, a yellow surface reflecting both middle and long wavelengths, a violet surface reflecting both short and long wavelengths or a white surface reflecting all wavelengths. In addition, information from any individual cone will be further confounded by the spectral characteristics of the illuminant. For example, the amount of illumination from blue sky light relative to direct sunlight changes throughout the day. As a result, the illuminant color can vary from blue to yellow^{125,285,338,386}. The ideal receptive fields for serving hue perception would be designed to help extract surface spectral reflectance independent of the illuminant. Here we discuss the features of theoretical receptive fields optimized to overcome the challenges associated with consistently signaling hue, independent of any underlying neural substrates.

Spectrally Opponent. Color vision is the ability to discriminate between different wavelengths, independent of intensity¹⁷³. Receptive fields with spectrally opponent interactions can extract wavelength information and thus carry color information^{286,267,53}. However, cone opponent receptive fields are not necessarily optimized for hue perception.

Spatially Coextensive. The first receptive field proposed to create a “pure color cell,” was the single opponent receptive field, which exhibits spectral opponency without any spatial opponency (**Figure 2.2A**). Also called spatially co-extensive or Type II^{384,63}, this receptive field provides color selectivity, the ability to extract spectral information unconfounded by spatial information. Spatially co-extensive, spectrally opponent receptive fields like **Figure 2.2A** would be theoretically color selective in that they respond to chromatic stimuli, but not achromatic patterns. However, these receptive fields act as simple wavelength detectors and cannot compensate for the changes in illuminant discussed above.

Double Opponency. To consistently signal hue, an optimal color-coding receptive field must compensate for the changes in illuminant discussed above. Double-opponent receptive fields, superimposing two opposing, spectrally and spatially opponent receptive fields (**Figure 2.2A**) have been proposed to help provide this color constancy^{79,108}. Double opponent receptive fields exploit the fact that, in the natural world, hue typically changes abruptly at object boundaries while illumination changes slowly across a visual scene. When the center receives light from the edge of an

object surface, some light falling in the surround is reflected from other objects in the scene under the same illuminant. If the illuminant changes to have more long-wavelength light, the increased L-cone stimulation in the center is opposed by greater L-cone stimulation in the surround, and ideally, the change in illumination is removed from the visual signal. Thus, double opponent receptive fields confer sensitivity to chromatic contrast at the edges of objects while remaining relatively insensitive to global changes in illumination.

Trichromatic. Normal humans are trichromats and a special requirement of optimal color coding for trichromats is that the receptive fields must compare all three cone types. This is because for neurons comparing only two out of the three cone types, a change in activity in the unsampled cone will not change the hue signaled by that neuron. For example, an L vs. M opponent neuron without S-cone input, as in **Figure 2.2A**, cannot discriminate between a red surface reflecting only long wavelengths and a violet surface reflecting both long and short wavelengths¹²⁹.

Low Spatial Resolution. If the ideal retina is composed of multiple types of feature detectors, spatial constraints must be considered, and the relative density of any one type should be no higher than required to serve its specific function. The color of a surface tends to be consistent all across it. Thus, in contrast to spatial vision, that requires a high density of detectors to capture the fine details of the shape of objects, hue detectors can accurately capture surface colors using a much lower resolution array of detectors. In summary, the ideal trichromatic hue-encoding system is a relatively sparse array of receptive fields with structures that are double-opponent and receive input from all three types of cones.

2.3.3 Interpreting Midget RGC Receptive Fields

Early models linking L vs. M midget RGCs to visual perception focused on either spatial or spectral opponency in isolation. Models focusing on their spectral opponency emphasized their potential role in encoding red and green hues. In contrast, models accounting only for achromatic spatial opponency lead to the perspective that spectral opponency is an unintended consequence of trichromacy and may be considered “poor engineering”²⁴¹.

Are Midget RGC Receptive Fields Optimal for Hue Perception? The earliest models followed the first parvocellular LGN (P cell) recordings^{87,384}, which have similar receptive field properties as their L vs. M midget RGC inputs. At the time, opponent process theory was still highly controversial¹⁷⁰ and the discovery of color-opponent neurons in the visual system was groundbreaking. The resulting hypothesis that the parvocellular LGN projections of midget RGCs are responsible for red-green hue perception arguably played a large role in shaping later research. Further, spatial opponency and the resulting responses to achromatic and spatially-structured stimuli were overlooked in many accounts of the physiological basis of hue perception.

In emphasizing, the proposed role of midget RGCs in mediating red-green hue percepts, it was argued that the optimal color-coding receptive field, was one in which an L-cone is surrounded entirely by M-cones, or vice versa. This receptive field, which would seem to require some cone-specific selective wiring, maximizes the spectral difference between the center and surround to maximally decorrelate the outputs of L- and M-cones’ overlapping spectral sensitivities (**Figure 2.1A**)^{38,280,347}. The “selective-wiring” model in **Figure 2.2B** was challenged by theoretical studies demonstrating that mixed L/M-cone receptive fields could generate sufficient spectral opponency^{286,224}. Though still debated by some^{214,389}, there is, at most, only a slight functional bias toward selective wiring^{41,121}.

A lack of selective wiring may be one argument against the idea that midget RGCs are optimized for hue perception. However, more importantly, from above, the ideal trichromatic hue-encoding system is a relatively sparse array of receptive fields with structures that are double-opponent and receive input from all three types of cones. The common L vs. M midget RGCs do not conform to any of these theoretical features of hue-encoding neurons. While our theoretical discussion cannot rule out a contribution to hue, we can conclude L vs. M midget RGCs, by themselves, are “non-optimal” for hue perception.

Are Midget RGC Receptive Fields Optimal for Spatial Vision? Near the fovea, the midget RGC’s receptive field center represents the cone providing direct input to the midget bipolar cell, while the surround is formed by feedback from horizontal cells contacting neighboring cones (**Figure 2.1C**)³⁶⁶. This feedback weights each cone’s response by the quantal catch in neighboring cones, essentially subtracting out the mean light level and allowing each individual cone feeding the center of midget RGCs to encode spatial contrast¹⁷⁶. In the central retina, midget RGCs set the limits of

human visual acuity³¹⁴.

Indeed, theoretical attempts to derive an optimal receptive field for the first step of spatial vision have all converged on the same circularly symmetric center-surround organization^{242,342,9}, often modeled as a Difference of Gaussians^{112,62,75}. As **Figure 2.2C** demonstrates, center-surround receptive fields are ideal edge detectors for encoding spatial contrast.

In contrast to early ideas emphasizing their putative role in color perception, more recent research into the evolution of the primate visual system provides a useful context for a modern understanding of L vs. M midget RGC function. Though sometimes compared to the X-cells of the mammalian retina, there is no true homolog to the midget circuit prior to prosimians²⁸⁷. The midget RGC circuitry evolved before uniform trichromacy²⁶⁴. In dichromats, for example, with only S- and L-cones, the midget RGC's antagonistic center-surround receptive field functions as an achromatic edge detector by comparing the input of a single L-cone to surrounding L-cones (**Figure 2.1D**).

Interim Conclusions. The receptive field structure of L vs. M midget RGCs is consistent with a role in edge detection. Their ability to respond to equiluminant edges defined only by wavelength differences makes visible forms that would be otherwise invisible. Spectral opponency can also increase the signal-to-noise ratio for edges defined by both intensity and wavelength. The idea that spectral opponency in L vs. M midget RGCs could enhance edge detection rather than contribute to color perception raises an important point. A response to wavelength changes does not imply a causal role in hue perception. As introduced above, hue perception requires detectors that will not respond to black-white edges.

In conclusion, while it may be arguable whether or not midget L vs. M RGCs are ideal achromatic encoders, it is indisputable that they are far from ideal for red-green hue encoding. This leaves two major unanswered questions: what is the physiological basis for hue perception and what role do midget RGCs play? Several different theories involving both the spectral and spatial aspects of midget RGC receptive fields have been proposed as tentative answers to this question. We next review the two main classes of explanation: multiplexing and parallel processing.

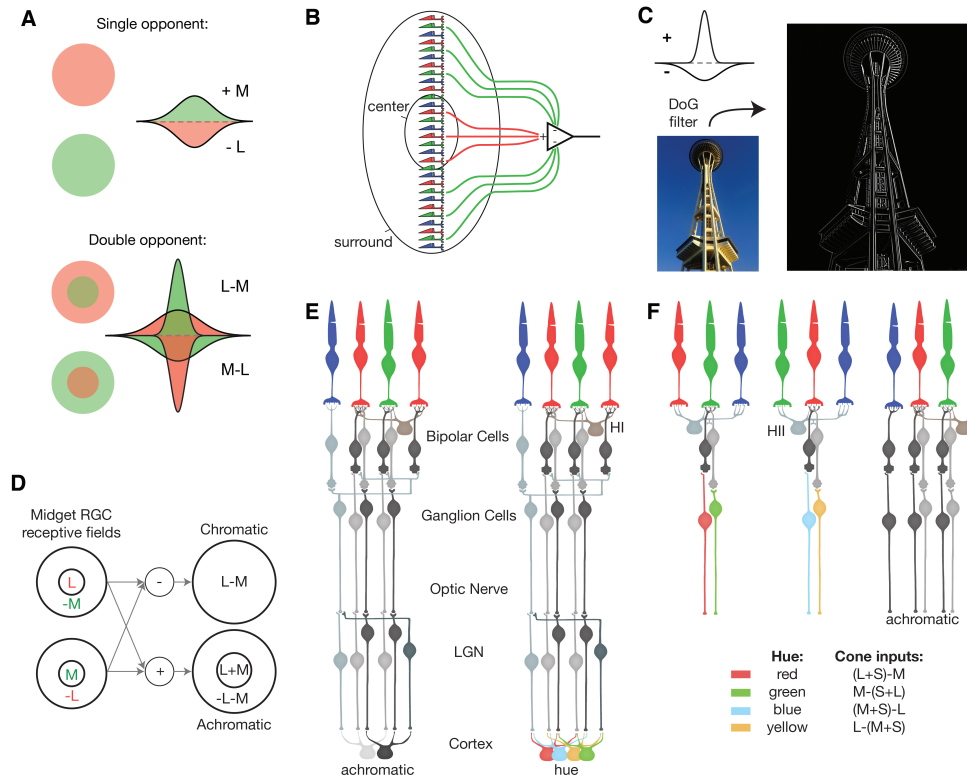


Figure 2.2 Models of receptive fields encoding form and color vision. (A) Diagrams of single and double opponent receptive fields. Adapted from Daw (1973)⁷⁹. (B) Putative receptive field formed by selective wiring. Adapted from Wiesel and Hubel (1966)³⁸⁴. (C) Edge detection performed by convolution of an image with an achromatic center-surround receptive field, or Difference of Gaussians filter. (D) Circuit for “de-multiplexing” chromatic and achromatic information from midget RGC receptive fields. Adapted from Derrico and Buchsbaum (1991)⁹⁵. (E) Circuit proposed by the multi-stage color model (De Valois and De Valois, 1993)⁸⁹. (F) Circuit proposed by the parallel processing model (Neitz and Neitz, 2016)²⁶⁸.

2.4 Multiplexing Models

The first class of models share the idea that each individual midget RGC does “double duty,” carrying information for both color vision and achromatic spatial vision, which are extracted by circuitry at higher levels of processing in the geniculostriate pathway. It has been said that red-green and black-white percepts are “de-multiplexed” by downstream circuits (Boycott and Wässle, 1999; Lennie and Movshon, 2005). The idea of multiplexing originated as an analog to attempts to efficiently compress chromatic and spatial information for color televisions (Ingling and Martinez-Uriegas, 1983; Derrico and Buchsbaum, 1991).

The most common models, summarized in **Figure 2.2D**, combine the outputs of midget RGCs to perform two main transforms: one to extract spectral information by removing spatial correlations

and another to extract achromatic spatial information by removing spectral information. The achromatic channels ($L + M$) sum L- and M-center midget RGC signals to serve as intensity contrast detectors. The putative chromatic channels (L vs. M) difference L-ON-center with M-ON center receptive fields to produce spatially coincident spectrally opponent receptive fields, as discussed above (**Figure 2.2A**). Accordingly, achromatic spatial structure will be absent in the chromatic channel, resulting in a low-pass chromatic filter, while the achromatic channel will retain the band-pass spatial tuning necessary for spatial vision.

A separate aspect of one of the best-known versions, the De Valois and De Valois (1993) multi-stage color model⁸⁹, was the need to reconcile the difference in cone inputs measured for L vs. M cone-opponent neurons and the opponent receptive fields required to account for hue perception, illustrated in **Figure 2.3A**. The four fundamental hue sensations are often assumed to represent the responses of four groups of hue-encoding neurons. Over the last 50 years, there have been different ideas about the exact nature of the cone inputs to the four fundamental hues. However, a convergence of modern evidence from experiments directly measuring hue perception indicate that all three cone types contribute to each hue in the following combinations: $L + S$ vs. M for red-green and $M + S$ vs. L for blue-yellow, respectively (**Figure 2.3A**)^{391,106,376,324}.

One of the great insights of the DeValois and DeValois model was that hue perception requires S-cone inputs to L vs. M opponent pathways^{391,106,376}. As an *ad hoc* solution to the discrepancy between L vs. M midget RGCs and the receptive fields required for hue perception, their multi-stage color model proposed that the necessary S-cone input to an L vs. M channel is accomplished by mixing in the outputs of S-cone opponent neurons (**Figure 2.2E**).

2.4.1 Evaluating the Double Duty Hypothesis

The DeValois and DeValois model was firmly based on the most recent anatomical, psychophysical and physiological results of the time, yet a number of assumptions were necessary where open questions remained. We can now revisit these assumptions in light of the research published in the 25 years since the multi-stage model was first proposed. One example is their explanation of how the required S-cone inputs from small bistratified RGCs are added in the process of building cortical receptive fields for hue perception. More recently, the classification of small bistratified RGCs as single opponent “pure color cells” has been called into question (compare **Figures 2.1E, 2.2A**,^{123,349}

but see⁶³). Thus, small bistratified RGCs and their S-ON projections may also confound spatial and spectral information. Moreover, the S-cone ON neurons were later identified as a part of the functionally distinct koniocellular pathway²⁴⁸ and there is no direct evidence for specific circuits combining signals from the koniocellular and parvocellular pathways.

While the theoretical L-M and L + M channels would decorrelate the outputs of midget RGCs, it has been argued that not all decorrelations are created equal²⁹² and the benefits depend on how these channels are implemented by neural circuitry. In general, however, asking a neuron to perform two jobs simultaneously has been said to ensure that both are done poorly³⁴³. Moreover, there don't appear to be any true modern examples of multiplexing RGCs involving two functions performed simultaneously. Perhaps the closest parallel is the fact that the same RGCs serve both photopic and scotopic vision, however, these functions are primarily performed separately under different conditions^{122,142}. Other examples of multiplexing RGCs involve one stimulus dimension modulating the encoding of another⁹⁴, however, this is different from two functions being encoded simultaneously.

The “de-multiplexing” multi-stage models are the result of speculation about the type of computation that would be required to produce selective detectors for wavelength and spatial contrast from combinations of spectrally opponent center-surround neurons, however, they lack firm experimental evidence from cortical physiology²¹⁸. They have also been criticized from an image compression standpoint, with the argument that decorrelation of chromatic and spatial information is best done early, ideally before transmission through the optic nerve⁹⁵. In contrast, an effort to test de-multiplexing models concluded the two dimensions cannot be disentangled in the early visual system¹⁸⁷. Moreover, the most successful models based on the “double duty” hypothesis do not make predictions about both spatial and spectral responses³⁰⁶.

The assumption that different aspects of color vision are all based on the same underlying neural substrates (e.g., L vs. M midget RGCs) has resulted in a tendency to expect the visual system to somehow extract hue information from the midget RGCs' receptive field output. However, the computational complexity required to separate chromatic from spatial information at subsequent stages of visual processing should not be underestimated. One higher stage is proposed to decorrelate spatial and spectral information, a second higher stage to add the required S-cone input (**Figure 2.2E**) and yet an additional stage, that has not been incorporated into current de-multiplexing

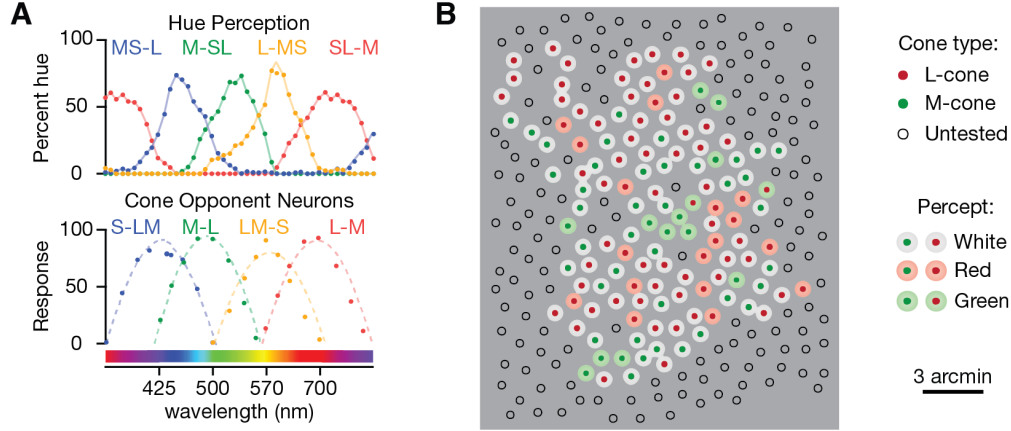


Figure 2.3 Experiments inspiring the revision of existing color models. (A) Spectral sensitivities and the corresponding cone inputs of the mechanisms responsible for red, green, blue, and yellow hues. The data was obtained from a hue scaling experiment, where participants report the percentage of red, green, blue, and yellow. Bottom: Averaged responses of color-opponent LGN neurons, which reflect their color-opponent RGC inputs. Both panels are replotted with wavelength units from De Valois (2004)⁸⁶. (B) Percepts associated with stimulating individual L- and M-cones in isolation may represent the responses of two types of individual midget RGCs, a larger group of achromatic contrast detectors and a smaller group that function as hue detectors. Adapted from Sabesan et al. (2016)³¹⁸.

models, to generate the double opponent receptive field structure required to create neurons that are able to contribute to invariant hue-encoding of spectral reflectance.

2.4.2 Multiplexing in the Light of Information Coding in the Retina

The need to compress RGC axons down to a 2 mm cable is often referred to as an “information bottleneck” within the visual system. Proponents of multiplexing models might claim superiority on this account: combining color and spatial information into one RGC could reduce the number axons in the optic nerve without reducing the transmission of information. Indeed, there are about six to seven million cones in a human eye and only about a million optic nerve fibers³⁴³. However, this represents the situation in the peripheral retina where convergent input from a large number of cones to each RGC results in a huge reduction in visual acuity relative to what could be supported by the cone mosaic. The loss of spatial information from this convergence is never recovered at higher levels in the visual pathway.

At the time multiplexing models were first proposed, a dominant view on the purpose of retinal function was to reduce redundancy and compress visual information to fit through the optic nerve, with the computations defining visual perception occurring in the cortex¹³. However, contrary to

the idea of information compression, in the fovea there is a divergence from cones to RGCs such that the ratio is about 3:1 RGCs:cone. Recent work in non-primate animal models has contributed to a growing appreciation for the diversity of RGC types^{371,11} and the sophisticated computations occurring within the retina^{135,383}. Even near the primate fovea, many of the at least 20 different RGC types are represented^{289,287}. What failed to be appreciated in the early work on the primate retina is that, with the exception of the midget RGCs, for which there are two for every cone (one ON and one OFF), each of the twenty or more RGC types represents a small percentage of the total. Thus, the retina is a massively parallel processing machine with many different types of RGCs carrying out diverse functions most of which operate at low spatial acuity and require only sparse representations. Thus, as discussed below, it seems plausible that, consistent with the current understanding of the plan of the retina, hue perception could be mediated by a relatively sparse set of RGCs that serve as hue detectors.

Recent considerations of the metabolic cost of information transmission have also questioned the efficiency of compressing information into a smaller set of RGCs, and revealed a more nuanced set of constraints defined not by the number of axons, but by their diameter. RGC axon diameters scale linearly with average firing rate^{290,291}. This relationship forms the basis of a law of diminishing returns – metabolic cost increases supralinearly with axon diameter while the information per spike falls as spike rate increases^{307,191}.

A population of parallel neurons, each carrying as much information as possible, is the most efficient coding scheme²¹¹. The midget RGC circuit, acting as an edge contrast detector, is already a model of energy-efficient parallel processing – each cone in the central retina contacts a single ON and OFF midget bipolar cell (**Figure 2.1C**). This allows baseline activity to remain low while the response ranges of each ON and OFF cell are devoted to signaling increments or decrements, respectively, in parallel²¹. Theoretically, multiplexing increments and decrements would double the information per axon, thus halving the number of axons while increasing axon diameter (and thus energetic cost) fourfold³⁴³. Taking these costs into account creates a strong pressure for more types of RGCs with thinner axons and lower spike rates, consistent with a parallel processing model.

2.5 Parallel Processing Models

L vs. M midget RGCs receptive fields are near optimal for high acuity spatial vision and are poorly suited for encoding hue. These facts plus the computational complexity required to separate hue from spatial information from L vs. M midget RGCs and a newer understanding of information processing in the retina has led to the suggestion of an alternative hypothesis: that the L vs. M midget RGCs’ only serve spatial vision – the function for which they are optimized – and they do not contribute to red-green hue perception. According to this idea, the front-end computations for hue perception are served, in parallel, by a second population of RGCs that have receptive field properties that are specifically optimized as hue detectors^{311,44,324,268}. The “pixel density” of the L vs. M midget RGCs is high to serve high spatial acuity but, as introduced above, the proposed parallel set of hue detectors need to be only relatively sparse to recover surface reflectance with much lower spatial acuity.

2.5.1 *Separate Subtypes of Midget Ganglion Cells for Hue and Spatial Vision*

If L vs. M midget RGCs mediate spatial vision, which RGCs encode color? To match the acuity of our hue perception, an undiscovered RGC type would need roughly the sampling density of the S-cone mosaic^{260,44}. The lack of alternative hue encoders makes midget RGCs an obvious candidate. We have proposed that the four fundamental hues are encoded by a small subset of L vs. M midget RGCs receiving input from neighboring S-cones (**Figure 2.2F**)³²⁴. The resulting L + S vs. M and M + S vs. L midget RGCs match the cone inputs for the four fundamental hues, as well as a population of rare RGCs^{83,81} and LGN neurons^{96,350}. These rare RGCs should not be ignored, as a potential hue-encoding RGC type needs to be only ~5–10% of foveal RGCs to match color acuity⁴⁴.

Each S-cone has a surround created by S-cone-preferring HII horizontal cells. Hue-encoding receptive fields are proposed to arise from the superposition of the S-cone center-surround receptive field with the L vs. M cone center-surround. These two are predicted to be combined by feedforward synapses²⁹⁶ from HII horizontal cells to L vs. M midget bipolar cells. The result simultaneously creates the S-cone input to L vs. M opponent cells and double opponency required to create nearly ideal hue-encoding RGCs (discussed in detail in Neitz and Neitz, 2016²⁶⁸). Indeed, computational

models of such color-coding midget RGCs can account for previously unexplained color phenomena, such as unique hues and variations in hue perception with L/M-cone ratios³²⁶.

Key strengths of this parallel processing hypothesis are its simplicity and specificity. All the key features of ideal hue-encoding neurons are proposed to be created in the retina simply by feed-forward from HII horizontal cells at the level of the bipolar cells in a single step as opposed to the idea of multiple stages at unspecified higher levels. The predicted mechanism for a parallel set of double opponent neurons includes specific cell types, neurotransmitters, and biophysical mechanisms²⁹⁶. While this level of detail may invite additional criticism, it also generates testable predictions that can be addressed by experiment. In contrast, the DeValois and DeValois model specified the computations for their “de-multiplexing” neurons, but not the underlying neural substrates.

2.5.2 Recent Research Supporting Parallel Processing Models

The parallel processing approach draws from the idea that each RGC’s receptive field acts as a feature detector, tuned to extract a specific type of visual information, such as direction, defocus, edges or hue. From this perspective, L vs. M midget RGCs that respond equally to red–green and black–white edges are not multiplexing, nor even confounding, red–green and black–white signals. Rather, they are reliably signaling a particular feature – the presence or absence of an edge. Accordingly, hue-encoding RGCs are signaling a different feature – the detection of a specific spectral reflectance distribution (**Figure 2.3A**). Importantly, these RGCs would not be directly responsible for percepts of hue and edges, but instead we are proposing that they serve as front-end mechanisms for making these computations.

A particularly influential line of evidence has been provided by high-precision psychophysics experiments enabled by the development of adaptive optics systems capable of delivering small spots of light while simultaneously imaging the underlying mosaic of cones¹⁵². Early experiments investigating spatial acuity found individual midget RGCs set the limit for spatial resolution³¹⁴. These results are inconsistent with models proposing midget RGC outputs are combined to “de-multiplex” color and spatial information. The loss of spatial information from the convergence in **Figure 2.2D** can never be recovered at higher levels in the visual pathway.

The unprecedented precision provided by adaptive optics imaging systems combined with recent advances in eye tracking and cone type classification³¹⁸ have enabled highly precise psychophysics

experiments investigating the percepts resulting from single cones (reviewed by Kling et al., 2019¹⁸⁸). The responses are highly consistent and reflect activity in the midget RGCs with single cone centers³²³. Consistent with parallel processing of hue and spatial information by separate types of midget RGCs, stimulation of most L/M-cones in the central retina results in percepts of white, with only a small subset eliciting color percepts (**Figure 2.3B**).^{318,325,322}. Further, the homogeneity of the surrounding cone type had no effect on which cones were associated with a perceived color, arguing against the idea that midget RGCs with strong L vs. M opponency serve hue perception. These experiments were the first to target stimuli to single cones of a known type and represent a major advance in linking perception to underlying neural substrates in awake, behaving humans and the results will undoubtedly continue to challenge long-held assumptions.

2.6 How Does the Cortex Use Wavelength Information?

Hue perception is just one of many functions that uses wavelength information. For example, the retina contains photopigments such as melanopsin and neuropsin, which carry additional wavelength information, but have no impact on the dimensionality of color vision^{167,40}. There are many examples of neurons carrying temporal, spatial or spectral information that is not extracted for visual perception, including color-opponent V1 neurons responding to chromatic stimuli that are not perceived^{147,179}.

In fact, many RGCs do not contribute to conscious perception at all, but instead mediate functions such as visually guided movements or circadian photoentrainment (for review, see Neitz and Neitz, 2016²⁶⁸). Wavelength information is extracted by several types of spectrally opponent RGCs for many functions other than color vision. For example, circadian rhythm photoentrainment and the pupillary light reflex are mediated by intrinsically photosensitive RGCs (reviewed in Do and Yau, 2010¹⁰²). Their receptive fields match the wavelength-encoding, single opponent receptive fields discussed above (**Figure 2.2A**)⁷³ – ideal for measuring the changes in chromaticity of ambient light throughout the day^{285,341} but they do not contribute to hue perception.

Several lines of evidence indicate that the ability to detect red-green edges is a distinct feature encoded separately from the ability to classify the appearance of lights as red or green. For example, patients with cerebral achromatopsia who suffer a total loss of hue perception, but still can detect chromatic borders, perceive shape from color and discriminate the direction in which colored

patterns move⁶¹. The existence of multiple mechanisms and uses for wavelength information also seems evident when comparing the cone inputs mediating color detection and color appearance. The studies identifying L + S vs. M and M + S vs. L as the cone inputs to hue perception measured color appearance^{391,106,376,326}. However, the classic psychophysical experiments that identified L vs. M and S vs. L + M as the “cardinal directions of color space”²⁰¹, measured detection. Krauskopf et al. (1982) noted the disparity between their cardinal directions and the red-green (L + S vs. M) and blue-yellow (M + S vs. L) hue axes of color appearance and later questioned the evidence for cardinal mechanisms²⁰⁰.

There is common ground between multiplexing and parallel processing models. In discussing the abundance of chromatic cortical neurons, DeValois and DeValois argue that only a few are responsible for the specification of color, while the majority instead use color information to specify the spatial (or other) characteristics of stimuli. A problem was a lack of agreement on which cells were relevant for hue perception. Though their proposed color transformations were not consistent with the majority of published cortical color tuning studies, DeValois and DeValois pointed out inconsistencies in the literature and claimed one could “cite some cortical study in support of (or against) almost any suggestion about cortical color processing”⁸⁹. We argue a similar situation exists today in the retina where different studies can be cited in support or against the existence of S-cone inputs to midget RGCs (for example, compare the cone opponency reported by De Monasterio and Gouras (1975)⁸³, Sun et al. (2006)³⁴⁷, and Field et al. (2010)¹²¹).

2.7 Future Directions

Both the parallel processing and multiplexing models would benefit from experiments linking the theories to their underlying neural substrates. However, an overarching difficulty for resolving the controversy over parallel vs. multiplexing theories is that each point of view reflects a deep-seated theoretical conviction. For those preferring the multiplexing view of L vs. M midget RGCs, “If the color signal is extractable, it makes little sense not to use it”²⁴. From a parallel processing standpoint, encoding color and spatial vision, two of the most fundamental aspects of visual perception, in a single binary channel makes little sense⁴⁴ and the information gained must outweigh the cost of extracting a color signal²¹².

Thus, further experiments to characterize the response properties of visual neurons alone are

not going to settle the controversy. Initial surveys of cone inputs to neurons in the retinal and LGN reported S-cone input to a subset of L vs. M neurons^{83,81,96} and later surveys confirmed these findings^{350,121}. However, skeptics of the parallel processing models favor a study by Sun et al. (2006) in which the authors recorded from a large population of midget RGCs and concluded S-cone input was unlikely³⁴⁷. An underlying problem is that the answers depend on how you ask the question. Results from receptive field measurements are a function of stimulus choice. For example, a full-field stimulus²¹⁶ may have reduced S-cone responses by driving the antagonistic S-cone surround receptive field mediated by HII horizontal cell feedback⁷². Indeed, the Sun et al. (2006) experiments did not detect S-OFF midget RGCs, despite a growing consensus that these neurons make up 5–10% of OFF midget RGCs in the macaque central retina^{189,121,358,282}. Taken together, these results further demonstrate the need to account for both the spatial and spectral dimensions of midget RGC receptive fields.

Consideration of underlying theoretical perspectives and stimulus biases will be essential for designing future experiments linking color vision models to their underlying neural substrates. Also, a broader perspective may help answer the larger questions about how our eye and brain process visual information. Hopefully, future research using cutting-edge technologies will provide satisfying explanations for long unanswered mysteries of vision.

CHAPTER 3

A Metamer for the Neural Circuits Mediating Hue Perception

3.1 Summary

Trichromatic color vision can be described by four fundamental, irreducible hue percepts, divided along two opponent channels: red-green and blue-yellow. In theory, trichromacy allows the underlying neural circuits three possible trichromatic cone-opponent comparisons of the cone outputs: L vs. M+S, M vs. L+S, S vs. L+M. Psychophysics have implicated the first two in red-green and blue-yellow hue perception, respectively, yet the third matches the most common trichromatic neuron in the early visual system. Here, we investigate the relationship between S vs. L+M neurons and hue perception. To overcome the technical difficulties in the past, we developed a psychophysics stimulator allowing unprecedented control over both the spatial and spectral aspects of the stimulus. We then utilized a well-known feature of trichromatic color vision, the perception of violet, to demonstrate conditions where large changes in S vs. L+M activity have no effect on hue perception. This work reveals a paradoxical failure of trichromacy by revealing conditions where wavelength information that is available at the cone mosaic is not extracted for hue perception.

3.2 Introduction

Color vision begins in the retina with comparisons between the three cone photoreceptors (long (L), medium (M) and short (S) wavelength sensitive) tuned to different regions of the visual spectrum. Yet the exact neural circuits responsible for combining the outputs of L-, M- and S-cones into the fundamental red, green, blue and yellow sensations remain one of the major unanswered questions in vision research. The retinal substrates for hue perception are classically thought to be “red-green”

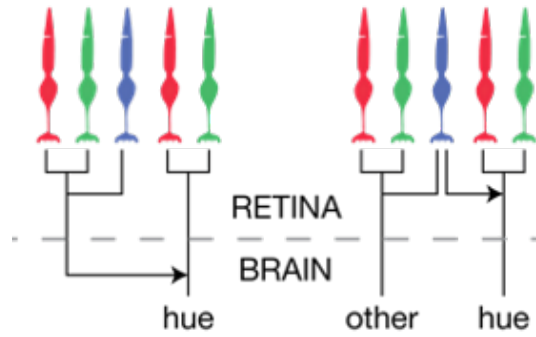


Figure 3.1 Models for obtaining neurons matching the fundamental hue sensations. Diagrams of the two models that propose how S-cone input can combine with L vs. M neurons to match the fundamental hue sensations and their involvement of the small bistratified ganglion cell (see text). The multi-stage color vision model is on the left and the midget ganglion cell model is on the right.

midget retinal ganglion cells (RGCs), which compare the outputs of L- and M-cones (L vs. M), and “blue-yellow” small bistratified RGCs that compare S-cones to the summed outputs of L- and M-cones (S vs. L+M)^{87,69}. However, the tendency to call these “red-green” and “blue-yellow” is misleading, as it only represents a hypothesized function, not unlike the use of “red”, “green” and “blue” cones for the L-, M- and S-cones, respectively. In fact, the cone inputs to these common cone-opponent neurons do not match the cone inputs to the four hues^{324,391}. Though small bistratified and midget ganglion cells are not the only cone-opponent ganglion cells^{82,73,282}, they are by far the most common and, as a result, the best studied. Thus, they have served as the cornerstone of modern color vision theories, despite significant discrepancies between their response properties and hue perception.

What is the exact relationship between the responses of the most common cone-opponent neurons and hue perception? The popular multi-stage model of color vision proposes the S-cone input carried by small bistratified RGCs is combined with the outputs of L vs. M midget RGCs in the cortex to create hue-encoding neurons matching the psychophysical axes⁸⁹. An alternative hypothesis does not involve the small bistratified RGC at all and instead proposes the involvement of a subset of L vs. M midget RGCs with S-cone input³²⁴ that match the cone inputs to both the psychophysical hues and a rare set of neurons in the early visual system^{82,350,96}. The two models, depicted in **Figure 3.1**, can be distinguished by their predictions regarding the percepts elicited by activity in the S vs. L+M neurons like the small bistratified RGC. If the S vs. L+M pathway contributes

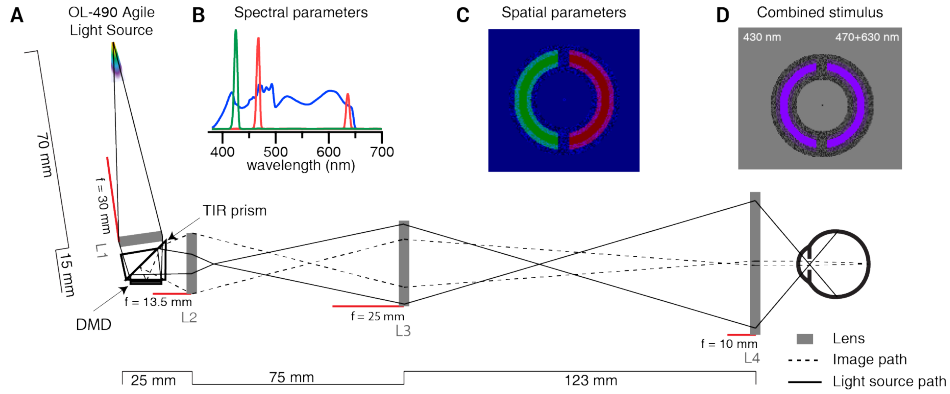


Figure 3.2 A novel psychophysics stimulator with unprecedented control over the spectral, spatial and temporal aspects of stimuli. (A) A programmable tunable light source (OL-490) defines the spectral distributions of three lights which replace the builtin “red”, “green” and “blue” LEDs of a DLP-based projector. The projector was integrated into an optical system to project an image of the digital micro-mirror device (DMD) onto the retina in the Maxwellian view. The DMD defines the spatial arrangement and temporal properties of the three lights. This combination allows fine control over the spatial, temporal and spectral properties of the final visual stimulus. (B) The spectra measured for the 430, 470 and 630 nm monochromatic lights and the “white” light used in the color match. (C) The spatial configuration of the color-matching stimulus. The monochromatic light is presented in the left half-annulus and the mixture is presented in the right half. (D) An illustration of the appearance of the final color-matching stimulus with the superimposed dynamic spatiotemporal noise mask.

to downstream hue-encoding neurons, stimuli that modulate S vs. L+M neurons will elicit hue percepts.

3.3 Results

Every color can be described as some combination of the four fundamental hue sensations³³¹. Trichromats generally agree that the color violet contains equal parts red and blue¹²⁹, and that red hues are perceived at both ends of the visual spectrum¹⁷⁷. As a result, a light perceived as violet can be obtained from either a monochromatic short-wavelength light or a mixture of two longer-wavelength lights that appear blue and red. This simple feature of color vision provides surprising insights into the retinal circuits underlying hue perception. Despite appearing similar, these two “violet” lights have very different spectral characteristics, which produce vastly different relative activations of the L-, M- and S-cones, and opposite responses from S vs. L+M neurons. If the S vs. L+M pathway plays a defining role in hue perception, these two lights should not appear identical. Though intuitive, this has never been tested conclusively.

Here, we formalized these observations about violet perception into a rigorous color-matching

experiment by testing whether participants could match a short-wavelength light to a mixture of longer-wavelength lights. As depicted in **Figure 3.2C**, the left half of a split-annulus was illuminated with a 430 nm test light and the right half-annulus was illuminated with a mixture of 470 and 630 nm lights. To trichromatic observers, the 430, 470 and 630 nm lights are perceived as violet, blue and red, respectively. The participants’ task was to adjust the amounts of the 470 and 630 nm lights to match the appearance of the 430 nm light.

There have been technical difficulties preventing these experiments in the past. The significant chromatic aberration produced by the 160 nm difference between the 470 and 630 nm lights results in “fringes” around the mixture, making color matching difficult. To solve this problem, we utilized a dynamic spatiotemporal noise mask, which overlapped the edges of the split-annulus used for color comparisons¹⁸¹. The annulus configuration was chosen to reduce the effects of macular pigment and other inhomogeneities associated with the fovea³³⁵.

Performing this experiment required an unprecedented level of control over the spatial, spectral and temporal aspects of the visual stimuli. The conventional optical systems used in color matching experiments lack the spatial and temporal control necessary for the dynamic noise mask. On the other hand, standard video technology lacks the precise control over wavelength required for color matching. Here, we developed a Maxwellian view visual stimulator combining the spatiotemporal resolution of a DLP light driver with the practically unlimited control over spectral parameters provided by a programmable tunable spectrum source (**Figure 3.2A**). With this technical innovation, we were able to experimentally confirm the long-standing observations about violet color perception and directly test the role of S vs. L+M activity in hue perception.

All subjects obtained precise matches between the violet 430 nm light and the mixture of the 470 and 630 nm lights. For each color match obtained, the spectral distributions of the 430 nm and 470+630 nm lights were measured with a spectroradiometer. **Figure 3.3A** shows the average spectral distribution of each light for one participant and the associated L-, M- and S-cone activities plotted in **Figure 3.3D** confirm the stimuli strongly differed in the relative S-cone to L/M-cone activity. Matches made by the two participants are plotted on the CIE 1976 u’v’ chromaticity diagram (**Figure 3.3C**). The chromaticity coordinates for each participant are tightly clustered, demonstrating a remarkable consistency in the color matches, which were performed over multiple days. As expected, the results for each participant do not overlap perfectly, reflecting individual

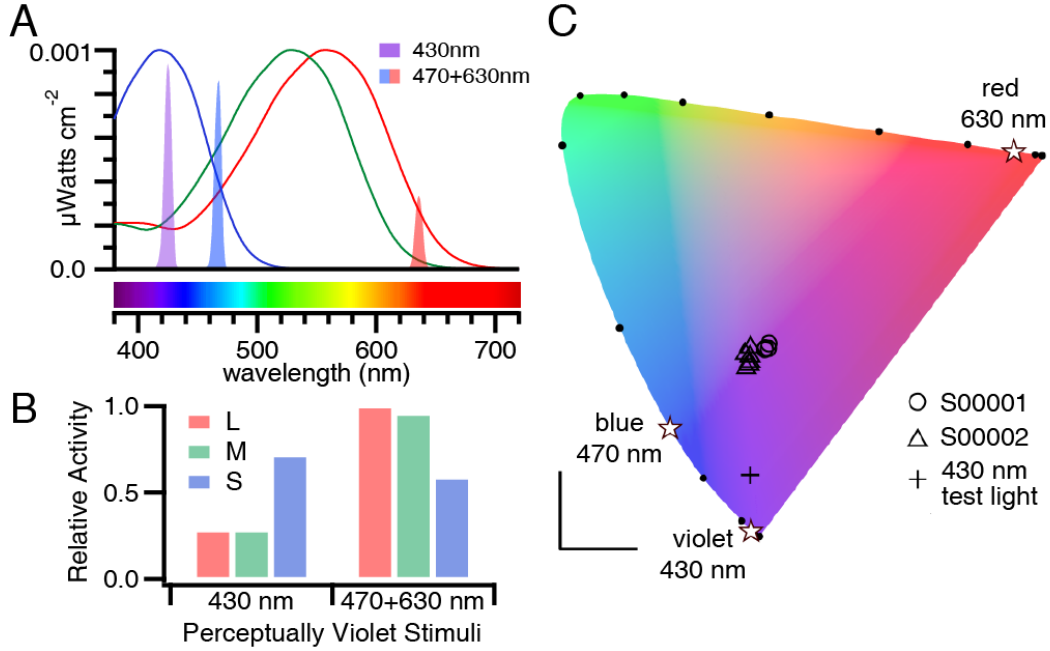


Figure 3.3 Activity in the S vs. L+M pathway is not associated with hue percepts (A) The average spectra of the 430 nm and 470+630 nm stimuli measured after each color match for S000001 ($n=6$). For reference, normalized L-, M- and S-cone spectral sensitivity curves are shown. **(B)** The relative L-, M- and S-cone activations elicited by the 430 nm and 470+630 nm lights after they were adjusted to match. **(C)** The chromaticity coordinates of the color matches for two participants displayed on a 1976 CIE $u'v'$ chromaticity diagram. Note the CIE diagram is based on measurements of cone activations, not hue perception.

differences in photopigments, L/M-cone ratios and pre-retinal filtering.

3.4 Conclusions

Here, we confirmed a well-known feature of trichromatic color vision: that identical violet percepts can be obtained by a monochromatic short-wavelength light or a mixture of two longer-wavelength lights perceived as red and blue. A systematic test of this observation has been prevented in the past by technical difficulties and was necessary because the implications of this phenomenon challenge widely-held assumptions about color perception.

The absence of a hue percept associated with the large differences in S vs. L+M pathway activity makes a strong argument against an essential role for the small bistratified RGC and other S vs. L+M neurons in hue perception in mediating hue perception. Moreover, this result underscores the need for caution in linking physiology to downstream visual functions³⁵³. Color perception is often the default hypothesis for spectrally-opponent neurons in the primate, however, a neuron's response to a chromatic stimulus alone is not causal evidence for a role in perceiving hue^{147,179}, especially at

the level of the retina.

More generally, our results challenge a common unspoken assumption in color vision research – that the cortex utilizes all available wavelength information for hue perception. **Figure 3.3D** demonstrates conditions where wavelength information is available at the level of the cone mosaic but does not reach the level of perception. However, the debate over the small bistratified ganglion cell’s contributions masks a much larger shortcoming in trichromatic hue encoding. As mentioned above, there are three possible trichromatic cone-opponent combinations, with two matching the cone inputs to red-green and blue-yellow hue perception (**Table 1**). If the visual system utilized all available wavelength information for hue perception, the small bistratified RGC would not just be *contributing* to hue perception. Instead, the small bistratified RGC would be *mediating* a third dimension of color vision, distinct from red-green and blue-yellow. Thus, the neural circuits mediating hue perception do not appear to take full advantage of trichromacy.

Cone Inputs	Percept
M vs. L+S	red-green
L vs. M+S	blue-yellow
S vs. L+M	???

In the classic color-matching experiments, any monochromatic light could be matched by adjusting the relative intensities of three well-chosen primary lights³⁴⁵. The matching stimuli were called metamers, lights with different spectral distributions that elicit the same relative activity in the L-, M- and S-cones and thus are indistinguishable to downstream neurons and at the level of perception³⁶⁸. Given the large differences in L-, M- and S-cone activations between the two lights shown in **Figure 3.3D**, the matching stimuli in our experiment are not metamers of the cone mosaic. However, the concept of a metamer is not limited to the cone photoreceptors and could be applied to any situation where information is lost because the degrees of freedom encoded by a set of neurons is less than the degrees of freedom of their input¹²⁶. Instead, the two violet stimuli are metamers of the post-receptoral color vision circuitry, producing identical levels of activity in the neurons responsible for encoding hue percepts.

Importantly, the idea that the neural circuits for hue perception do not take full advantage of trichromacy is not in conflict with the classic color-matching experiments that first demonstrated

that color vision is based on three cone types³⁴⁵. Rather our results are consistent with the idea that dimensionality of hue perception is determined not only by the number of receptors, but also by the circuits processing their outputs¹⁷³. Although all three cones are used to perceive red, green, blue and yellow, hue perception does not utilize all three possible cone-opponent pathways (**Table 1**). What is remarkable about the results presented here is that the circuits extracting S vs. L+M *are* available – in fact, the small bistratified RGC is the most common trichromatic cone-opponent neuron in the early visual system and there are at least five RGCs carrying this spectral signal⁶⁹.

The visual system presents many counterintuitive results and paradoxes^{20,1,256}, which provide invaluable testing grounds for color vision theories. The violet color matching experiment here offers an exciting new opportunity for progress towards a satisfying account of the neural substrates of hue perception, while our psychophysics stimulator offers the stimulus control necessary to test many predictions of color vision theories. The concept of metamerism elegantly deduced trichromacy long before the identities of the cone photoreceptors were known. Post-receptoral metamers, like the violet stimuli described here, may provide similar insights for the neural circuits mediating hue perception.

3.5 Methods

3.5.1 Participants

Two female participants aged 25 and 26 years old with normal color vision participated in this study. The 470 nm light appeared unique blue against a black background for both participants. The participants also confirmed that the background appeared white or gray, without any hue sensation.

3.5.2 Stimulator

A newly-developed Maxwellian view spectro-spatio-temporal visual stimulus generator was used in the color-matching experiments (see **Figure 3.2**, Kuchenbecker et al., in preparation). The stimulator combined a Gooch & Housego OLM-490 with a LightCrafter DLP 4500 (Texas Instruments). The strength, peak wavelength and full-width half-max of the spectral aspect of the stimulus were specified using a custom user interface to the OLM-490 API. Multiple spectral distributions could be specified independently and then combined into a single stimulus. Thus, the 470 + 630 nm

mixture could be produced as a single light but treated as two monochromatic lights at 470 nm and 630 nm (both with a 10 nm full-width at half-max) with independently adjustable intensities.

To combine the spectral control of the OLM-490 with the The "red", "green" and "blue" channels of the LED are presented sequentially at 180 Hz. The built-in LEDs were replaced with the OLM-490 and the temporal presentation of the three spectral distributions in **Figure 3.2B** was synchronized with the LightCrafter's update rate.

The spatial and temporal aspects of the stimulus were programmed using the p5.js JavaScript library and demonstrations will be made available upon publication. To minimize influence of the macular pigment, the wavelengths were presented as a split annulus¹⁸¹ with an inside diameter of 6 degrees and an outside diameter of 7 degrees. A random-dot pattern of white noise was positioned such that it overlapped the edges of the split annulus and was updated at 30 Hz. This masked the fridge effects resulting from chromatic aberration associated with the mixture of the 470 and 640 nm lights.

3.5.3 Experiment

The participants were asked to adjust the relative amounts of the 470 nm and 630 nm lights to obtain a hue match to the 430 nm light, and then adjust the intensity of the fixed 470 nm and 640nm ratio to achieve a brightness and saturation match. The participants could fine-tune their settings until they were satisfied with their match to the 430 nm light. Participants were instructed to fixate at the center of the split-annulus during the experiment. The matching experiment was performed multiple times over several days and the starting intensities of the 470 nm and 630 nm lights was randomized for each trial.

The spectral distributions of the stimuli for each match was measured by a spectroradiometer (Konica Minolta CS-2000). The quantal catches in **Figure 3.3B** associated with the stimuli were obtained by integrating the spectral distributions with the corneal sensitivities of the L-, M- and S-cones, obtained with the Neitz photopigment template. The corneal sensitivities assumed peak wavelengths of 559, 530 and 419 nm and optical densities of 0.4, 0.38 and 0.33 for the L-, M- and S-cones, respectively.

CHAPTER 4

An S-cone Circuit for Edge Detection in the Primate Retina

The study presented in this chapter has been published as:

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4.1 Summary

Midget retinal ganglion cells (RGCs) are the most common RGC type in the primate retina. Their responses have been proposed to mediate both color and spatial vision, yet the specific links between midget RGC responses and visual perception are unclear. Previous research on the dual roles of midget RGCs has focused on those comparing long (L) vs. middle (M) wavelength sensitive cones. However, there is evidence for several other rare midget RGC subtypes receiving S-cone input, but their role in color and spatial vision is uncertain. Here, we confirm the existence of the single S-cone center OFF midget RGC circuit in the central retina of macaque monkey both structurally and functionally. We investigated the receptive field properties of the S-OFF midget circuit with single cell electrophysiology and 3D electron microscopy reconstructions of the upstream circuitry. Like the well-studied L vs. M midget RGCs, the S-OFF midget RGCs have a center-surround receptive field consistent with a role in spatial vision. While spectral opponency in a primate RGC is classically assumed to contribute to hue perception, a role supporting edge detection is more consistent with the S-OFF midget RGC receptive field structure and studies of hue perception.

4.2 Introduction

Anatomical evidence for S-OFF midget RGCs is seen in the macaque (Old World) monkeys^{189,198,358} (but see Kolb et al.¹⁹³) but not in New World monkeys^{217,221}. Multi-electrode recordings in the macaque far peripheral retina find weak S-cone input to OFF midget RGCs, consistent with non-selective input from S-OFF and L/M-OFF midget bipolar cells¹²¹; however, pure S-cone center, OFF midget RGCs have remained elusive in single cell electrophysiology^{236,80,347} and electroretinography^{202,203}. The discrepancies in evidence for S-OFF midget RGCs may be due to differences in species²⁴³, retinal eccentricity and methodology, thus, here, we sought both anatomical and physiological confirmation in the macaque retina. Because color vision²⁶⁰, spatial acuity³⁵⁷ and midget RGC response properties vary considerably with eccentricity³³², we focused our efforts on the central retina where our research is most relevant to human visual perception.

The existence and function of S-OFF midget RGCs is relevant to a major unsolved question: how and where color and spatial information are separated in the visual pathway. This has been proposed to be accomplished in the cortex by theoretical downstream “de-multiplexing” circuits^{89,95} (but see Kingdom & Mullen¹⁸⁷) or in the retina by separate populations of RGCs for color and spatial information^{311,44,324}. In the central retina, each L- and M-cone provides the sole direct input to an ON and OFF midget circuit, forming a “private line” pathway from single cones to the parvocellular lateral geniculate nucleus (LGN)¹⁹⁵. Their center-surround receptive fields perform a simple computation: comparing a single L- or M-cone center to neighboring L/M-cones in the surround. However, this seemingly simple computation introduces a complication for neural coding that vision science has yet to resolve. The center and surround receptive fields differ in both spatial location and spectral sensitivity, creating a neuron with both spatial and spectral opponency. The result is that L vs. M midget RGCs carry both spatial and spectral information, but confound the two such that from an individual midget RGC’s spike output, downstream neurons cannot distinguish between chromatic and spatial stimuli (see²⁸⁴). The goal of this work is not only to confirm the existence of S-OFF midget RGCs but understand the details of their circuitry and receptive field properties so they can be fit into a larger understanding of the function of midget RGCs in color and spatial vision.

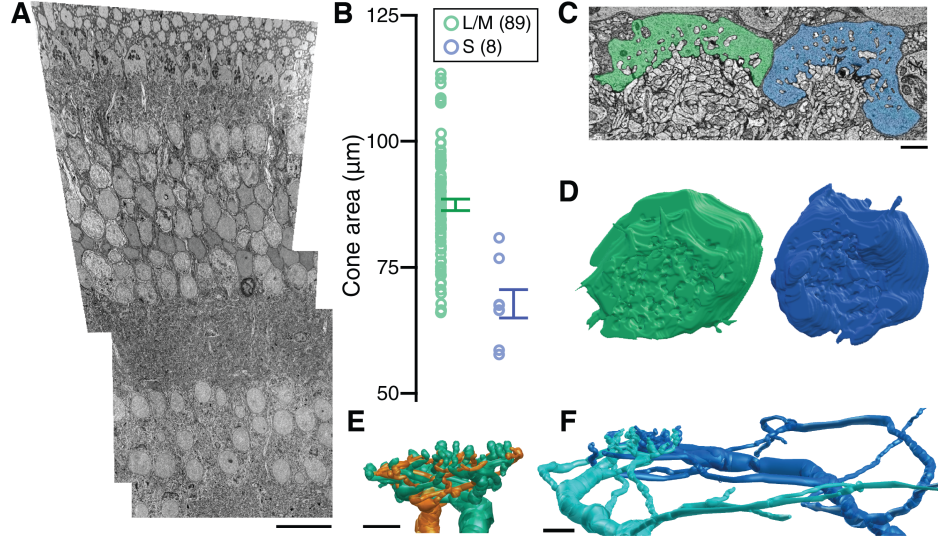


Figure 4.1 Identification of L/M- and S-cones using serial EM. (A) Transmission EM image of the block of tissue (magnification 2000x). (B) Area of L/M- and S-cone pedicles (S: $67.705 \mu\text{m}^2 \pm 2.81$, LM: $87.359 \mu\text{m}^2 \pm 1.126$; $p = 0.0013$). (C) Electron micrograph of neighboring LM- (green) and S-cones (blue). Scale bar is $2 \mu\text{m}$ (D) 3D reconstructions of neighboring S- and L/M-cones (blue, green). (E) 3D reconstruction of L/M-ON (teal) and L/M-OFF (orange) midget bipolar cell dendrites at an L/M-cone. (F) 3D reconstruction of S-ON bipolar cell dendrites at an S-cone.

4.3 Results

4.3.1 S-cone circuits of the outer retina

To confirm the existence of S-OFF midget RGCs using serial electron microscopy (EM; **Figure 4.1A**), a first step was to reliably identify S-cones. We identified candidate S-cones by their small size and lack of long telodendria, branches forming gap junctions with neighboring cones¹⁹³. While S-cones were indeed smaller than L/M-cones, distinguishing the two at any single section was difficult, especially given the changing landscape of the foveal slope in our sample (**Figure 4.1B**, **4.1C**, **4.1D**).

Candidate S-cones were verified by reconstructing post-synaptic neurons. Cones signal changes in photon catch by modulating the rate of glutamate release from ribbon synapses onto a post-synaptic “triad” consisting of an ON-bipolar cell and two horizontal cells (reviewed by Sterling & Matthews³⁴⁴). L/M- and S-cones contact stereotyped horizontal cell and ON bipolar cell subtypes, allowing unambiguous confirmation of cone type by reconstructing the outer retina circuitry.

In the central retina, L/M-cones are densely innervated by a single ON midget bipolar cell while S-cones provide input to several S-ON bipolar cells (**Figure 4.1E**, **4.1F**)^{198,358,372}. Furthermore, S-

ON bipolar cells contact multiple S-cones, forming the distinctive lateral branches shown in **Figure 4.2C**. We reconstructed 14 S-ON bipolar cells (**Figure 4.2A**), each contacting up to three S-cones but receiving the majority of their input from a single S-cone.

The primate retina contains two horizontal cell subtypes: HII horizontal cells preferentially contact S-cones while HI horizontal cells avoid S-cones entirely^{2,137,51}. We reconstructed both types (**Figure 4.2B**) and confirmed each S-cone was densely innervated by HII, but not HI horizontal cells. In past light microscopy experiments, the horizontal cell subtypes could be distinguished by their dendritic field size and cone contacts, however, applying these features to serial EM experiments requires considerable annotation efforts. To date, no complete serial EM reconstructions of primate horizontal cells have been published. We developed two criteria for early identification of horizontal cell subtypes: soma size and primary dendrite diameter (**Figure 4.2D**). The horizontal cells identified using these criteria matched established morphological descriptions from light microscopy^{31,192}.

4.3.2 Each S-cone in the central retina provides the sole input to an OFF midget circuit.

Having identified eight S-cones by morphology and postsynaptic circuitry, we next searched for OFF midget bipolar cell contacts. Indeed, a single OFF midget bipolar cell contacted each S-cone, as shown in **Figure 4.3B**. To verify the transmission pathway from S-cones to OFF midget RGCs, S-OFF bipolar cell contacts to S-cones were characterized. We next verified that S-OFF bipolar cells provide the sole input to OFF midget RGCs and confirmed the S-cone signal is not diluted by L/M-cone input from multiple midget bipolar cells, as seen in the peripheral retina^{121,359}. Six OFF midget bipolar cells contacting neighboring L/M-cones were used as controls.

Each OFF bipolar cell subtype is located at a characteristic distance from the ribbon synapse (**Figure 4.3C**)^{45,166,30}. At L/M-cones, OFF midget bipolar cell contacts are located at the “triad associated” position, adjacent to the membrane invaginations containing horizontal cell and ON bipolar cell dendrites. Virtually all S-OFF midget bipolar cell synapses were found in the same location, adjacent to the membrane invaginations containing S-ON bipolar cell dendrites (**Figure 4.3C**). This distinguished the S-OFF midget bipolar cell dendrites from the thin, straight, OFF diffuse bipolar cell dendrites making basal contacts around the edges of the S-cone pedicle, as in **Figure 4.3A**. The distance from the ribbon synapse shapes bipolar cell responses by defining the

timing and concentration of available glutamate^{303,98}. Thus, S-OFF midget bipolar cells likely have similar response properties to L/M-OFF midget bipolar cells.

Basal contacts between cone pedicles and OFF bipolar cells are recognized as membrane densities without associated pre-synaptic vesicles. The basal contacts were quantified at two S-cones, where S-OFF midget bipolar cells made 22 and 23 basal contacts. The basal contact counts are likely underestimates as the 90 nm section thickness prevented exhaustive tracing of all bipolar cell dendrites through the S-cone pedicle. However, these numbers are comparable to Klug et al.¹⁸⁹, and to the 25 OFF midget bipolar cell basal contacts counted at a neighboring L/M-cone.

Near the fovea, each midget bipolar cell contacts a single midget RGC, forming a “private line” from a single cone to the parvocellular LGN. Outside the central retina, the number of bipolar cells converging on a single midget RGC scales with eccentricity^{76,195}. We traced seven of the S-OFF midget bipolar cells to the inner retina, where each contacted a single OFF midget RGC with an average of 40 ribbon synapses (**Figure 4.3D, 4.3E, 4.3G**). The eighth S-OFF midget bipolar cell ran off the edge of the volume. Ribbon synapses from S-OFF midget bipolar cells onto other RGCs were rarely observed.

Like the S-OFF midget bipolar cells, each L/M-OFF midget bipolar cell provided the sole input to an OFF midget RGC. Ribbon synapse counts have been reported to divide L/M-OFF midget bipolar cells into two populations, referred to as “sparsely” and “densely” branching, which presumably correspond to L- and M-cones^{43,42,319}. The L/M-OFF midget bipolar cells in our study made an average of 39 or 51 ribbon synapses, with the sparsely branching group comparable to the S-OFF midget bipolar cells (**Figure 4.3G**). The only anatomical difference was that S-OFF midget bipolar cells had slightly smaller somas than the LM-OFF midget bipolar cells (**Figure 4.3F**).

Overall, we found no anatomical evidence for a significant functional difference between L/M-OFF and S-OFF midget circuits. Taken together, our reconstructions indicate the LGN indeed receives input from single S-cones through S-OFF midget RGCs in the central retina.

4.3.3 S-OFF midget ganglion cells encode spatial information with center-surround receptive fields

During the course of a larger series of electrophysiology experiments on S-cone inputs to midget RGCs using our custom-built light source (see **Methods**), we encountered four midget RGCs with

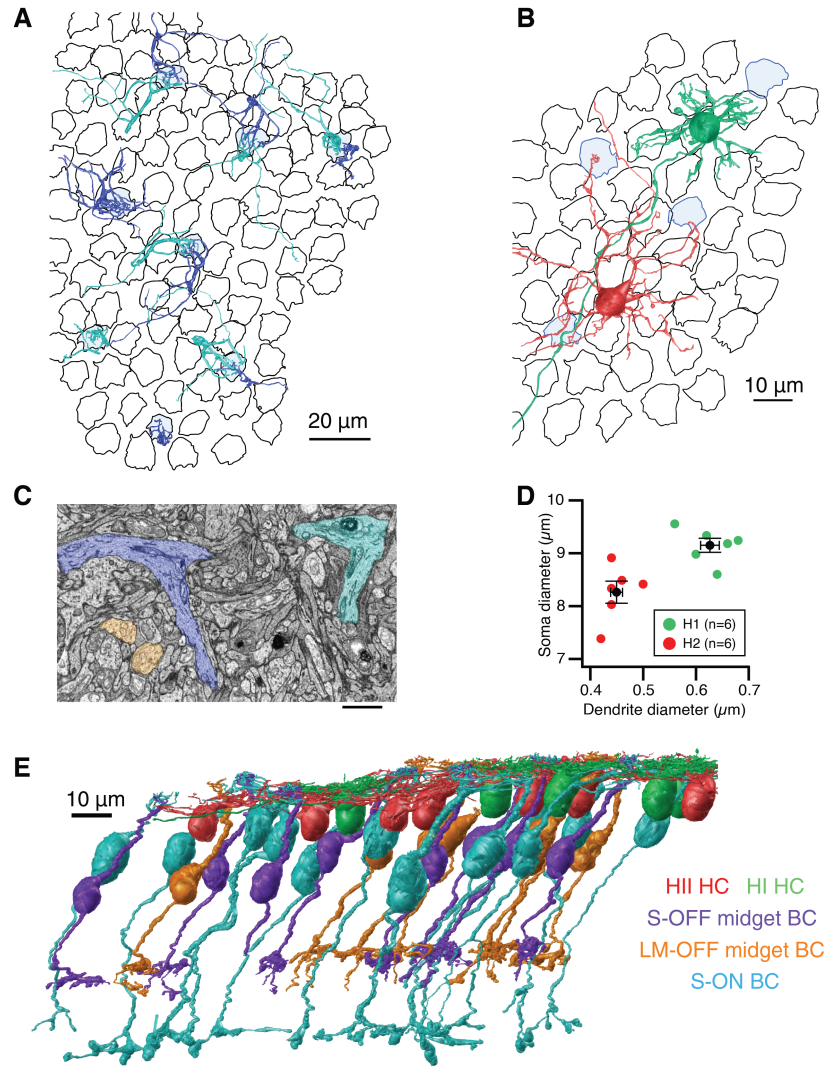


Figure 4.2 Serial EM reconstruction of the S-cone circuitry in macaque central retina. (A) 3D reconstructions of the dendrites of 14 S-ON bipolar cells over the cone mosaic (S-cones in blue). (B) 3D reconstructions demonstrate the morphological differences between HI (green) and HII (red) horizontal cells (S-cones in blue; L/M-cones in black). (C) Two S-ON bipolar cell dendrites (blue, cyan) converge towards an S-cone. These lateral branches distinguish S-ON bipolar cells from ON midget bipolar cells (orange) which branch directly beneath the L/M-cone pedicle. Scale bar is 2 μm . (D) Soma diameter plotted against primary dendrite diameter for HI and HII horizontal cells ($n = 6$). The dendrite diameters are $0.627 \mu\text{m} \pm 0.018$ and $0.450 \mu\text{m} \pm 0.011$ for HI and HII horizontal cells, respectively ($p \leq 0.001$). Soma diameters were $9.148 \mu\text{m} \pm 0.134$ and $8.259 \mu\text{m} \pm 0.211$ for HI and HII horizontal cells, respectively. (E) 3D reconstructions of the retinal neurons in this study.

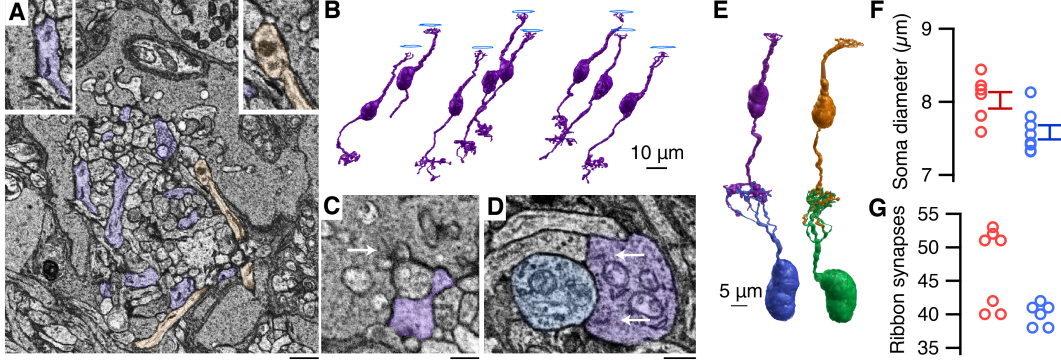


Figure 4.3 Each S-cone in the central retina provides the sole input to an OFF midget circuit. (A) S-OFF midget (purple) and OFF diffuse (orange) bipolar cell processes at an S-cone. Left inset: An S-OFF midget bipolar cell basal contact. Right inset: An OFF diffuse bipolar cell basal contact. (B) 3D reconstructions of the S-OFF midget bipolar cells contacting eight S-cones. (C) An S-OFF midget bipolar cell dendrite at the triad-associated position. Scale bar is 500 nm. (D) An S-OFF midget bipolar cell circuit (left) with an L/M-cone OFF midget circuit (right). (E) Comparison of an S-cone OFF midget bipolar cell circuit (left) with an L/M-cone OFF midget circuit (right). (F) Soma diameters for S- and L/M-cone OFF midget bipolar cells (S: $8.02 \mu\text{m} \pm 0.11$, $n = 7$; LM: $7.93 \mu\text{m} \pm 0.36$, $n = 9$, $p = 0.0175$). (G) Ribbon synapses between OFF midget bipolar cells and OFF midget RGCs. S-OFF midget bipolar cells made 40 ± 0.683 ribbon synapses. LM-OFF midget bipolar cells formed two groups of 39.67 ± 0.577 ($n = 3$) and 51.75 ± 1.291 ($n = 4$) ribbon synapses (expressed as Mean $\pm$ SD). The probability of obtaining these two groups by chance from a single normally-distributed group was estimated using a bootstrap procedure ($p = 0.0083$, see Methods).

S-OFF responses and single cone receptive field centers (**Figure 4.4B**). A temporally-modulated S-cone isolating square-wave was used to assess S-cone inputs for every RGC encountered. The S-cone isolating stimulus modulates S-cone activity while holding L- and M-cones constant¹¹⁴. We experimentally validated the S-cone isolating stimulus at the beginning of each experiment by ensuring parasol RGCs did not respond to S-cone isolating modulations (**Figure 4.4A**). As shown in **Figure 4.4B**, each candidate S-OFF midget RGC responded robustly to the S-cone decrements. The tonic responses in **Figure 4.4B** are characteristic of OFF midget RGCs and distinct from other cell types, such as the OFF parasol RGC in **Figure 4.4A**.

The S-cone response kinetics were characterized using a time-varying “white noise” stimulus where the S-cone contrast of each frame was drawn pseudo-randomly from a Gaussian distribution (mean = 50%, SD = 30%, **Figure 4.4C**). Convolving the stimulus with the elicited spike rate histogram in the Fourier domain returns a “filter” that captures the linear components of the response. In other words, the linear filter represents the average S-cone modulation during the time preceding a spike and is proportional to the neuron’s impulse response function^{56,308}. The average

negative peak at 25 ms in **Figure 4.4C** indicates that the neuron fires following decrements in S-cone contrast, the defining characteristic of an “S-OFF” neuron.

Our anatomical data confirm that HII horizontal cells carry both L/M- and S-cone signals (**Figure 4.2B**)^{137,72}. While an HII horizontal cell mediated L/M-cone surround has been reported in individual S-cones²⁷⁸, feedback from the strong S-cone surround has not been reported. Consistent with the presence of HII horizontal cell-mediated S-cone feedback, the S-OFF midget RGCs responded weakly to large, full-field S-cone isolating stimuli, preferring small spots centered over the receptive field (**Figure 4.5A**). The weak responses to full-field stimuli may help explain why previous electrophysiology studies did not find evidence of S-OFF midget RGCs³⁴⁷.

The center-surround receptive field structure was investigated using an expanding spot stimulus temporally modulated in luminance (**Figure 4.5B**). The spike rate increased as the spot size expanded to cover the entire center receptive field, then began decreasing as the spot further expanded to cover more of the antagonistic surround receptive field. The tuning profile of spatial opponency is comparable to L/M-cone OFF midget RGCs and clearly distinguished S-OFF midget RGCs from melanopsin RGCs, the other known S-OFF neuron in the primate retina⁷³. Taken together, the S-OFF midget RGC anatomy and physiology indicates a center-surround receptive field, similar to L/M-OFF midget RGCs (**Figures 4.4C, 4.5A–D**).

The relationship between center-surround receptive fields, spatial information and edge detection is well-established^{242,176,292,342}. Thus, the presence of center-surround structure in S-OFF midget RGCs indicates that, regardless of spectral tuning, the S-OFF midget RGC is carrying spatial information.

4.4 Conclusions

Here, we resolve a long-standing controversy and confirm the existence of S-OFF midget RGCs in the macaque retina. In addition, our work provides the most comprehensive reconstruction of the primate outer retinal S-cone circuitry to date, including the first complete reconstructions of horizontal cells in the primate retina.

We find that every cone in the central retina provides the sole input to an OFF midget bipolar cell. The pure S-cone midget RGC centers are not observed in the peripheral retina. There, OFF midget RGCs collect input from all nearby OFF midget bipolar cells, regardless of their cone

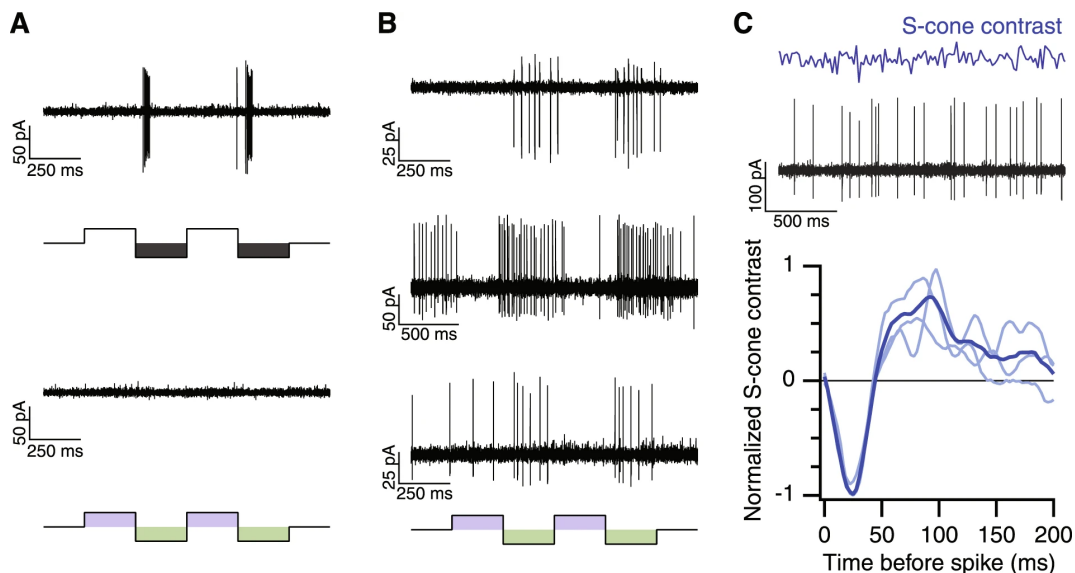


Figure 4.4 Spike responses of S-OFF midget RGCs. (A) Responses of an OFF parasol RGC to temporally-modulated luminance and S-cone isolating spots. (B) Responses of three OFF midget RGCs to temporally-modulated S-cone isolating spots. (C) An example of the S-cone isolating Gaussian white noise (top) an S-OFF midget RGC's response (middle). Bottom: The average linear filter (blue) from three S-OFF midget RGCs (light blue). The linear filter represents the average S-cone contrast preceding a spike.

contacts^{358,121}. We propose that S-OFF midget RGCs should not be considered distinct from L-OFF and M-OFF midget RGCs. In the central retina, OFF midget bipolar cells draw indiscriminately from single cones regardless of type and, in the peripheral retina, where each midget RGC draws from multiple midget bipolar cells, the centers become mixed.

We find that S-OFF midget RGCs have the same center-surround receptive field structure as L vs. M midget RGCs. This receptive field structure has been characterized as optimal for the edge detection required for spatial vision²⁴². It has been argued that edge detection must be achromatic and any degree of spectral opponency is detrimental^{241,24}. However, not all edges are defined by changes in intensity alone, and equiluminant edges are common in natural scenes¹⁵¹. Thus, the spectral opponency in S-OFF midget RGCs could be used to signal the presence of an edge defined not only by a change in intensity, but also wavelength (reviewed by Patterson et al.²⁸⁴). S-cones contribute to the detection of white-yellow and gray-brown boundaries³⁹, and are necessary when these boundaries are equiluminant. Both involve S-cone decrements, so S-OFF midget RGCs are well-suited for signaling brown or yellow objects against neutral backgrounds. In addition, seeing gray objects against the blue sky could be mediated by S-OFF RGCs.

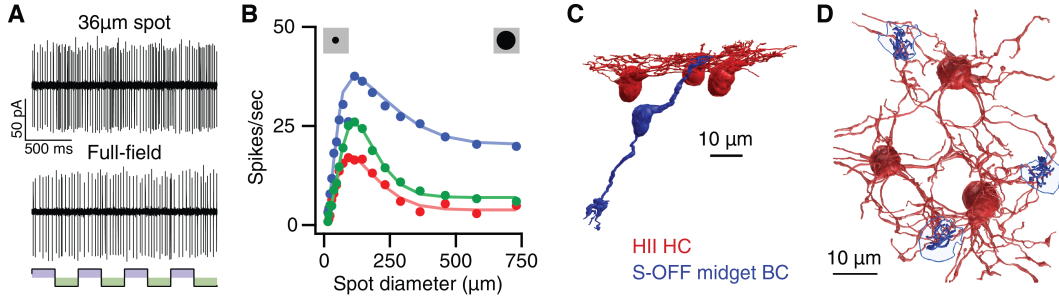


Figure 4.5 S-OFF midget RGCs encode spatial information with a center-surround receptive field. (A) Responses of an S-OFF midget RGC to a 36 μm spot vs a full-field stimulus. (B) Responses of an S-OFF midget RGC (blue) and two control L/M-cone midget RGCs (red and green) to achromatic spots of increasing diameter presented as temporal modulations (4 Hz square-wave). Smooth curves are fits to a Difference of Gaussians model. (C) The S-OFF midget receptive field obtained by the Difference of Gaussians fit in B. (D) The anatomical basis for the S-cone center-surround receptive field. The center receptive field represents the single S-cone input directly to the S-OFF midget bipolar cell (blue). HII horizontal cell feedback (red) forms the antagonistic surround receptive field. Scale bar is 10 μm .

A role in color perception is often the default hypothesis for cone-opponent neurons, particularly those with S-cone input. Indeed, S-OFF midget RGCs have been proposed to mediate yellow hue percepts, forming the OFF counterpart to the small bistratified RGC⁷⁰, despite extreme asymmetries between the two types. However, there is no evidence that the color and spatial information from OFF-midget RGC are ever separated in our visual system. S-OFF midget RGCs may have evolved only to signal edges of objects against their background, particularly edges principally defined by spectral contrast between short and long wavelengths and they may have no role in generating color percepts.

Moreover, the S vs. L+M spectral tuning in both small bistratified and S-OFF midget RGCs does not match the cone inputs to blue-yellow hue perception^{380,346,86,376,322,106} and a subset of neurons with cone inputs matching the fundamental hues^{83,81,350,96} have been proposed to mediate hue perception instead (for review, see Neitz & Neitz²⁶⁸). The L vs. M midget RGCs with S-cone input are another controversial midget RGC subtype that warrants additional investigation. The significant technical challenges involved in studying rare S-cone inputs to parafoveal midget RGCs that may have limited these investigations in the past may now be overcome with the multidisciplinary approach described here.

4.5 Methods

All methods were performed in accordance with the relevant guidelines and regulations.

4.5.1 Serial Electron Microscopy

Microscopy. The tissue was imaged using a Zeiss Sigma VP field emission scanning electron microscope equipped with a 3View system and sectioned in the horizontal plane. In 2018, the system was updated with an OnPointTM detector that has been optimized for backscattered electrons (BSE) (Gatan, Inc.). All the EM images were obtained with the new detector. Optimized for biological samples, the OnPointTM detector provides high BSE collection efficiency which translates to a favorable signal-to-noise ratio for visualizing small, low contrast features such as synaptic ribbons that have previously been a challenge for Serial block-face scanning. In each 90 nm section, an area approximately 200 μm on a side was imaged as a 5 X 5 montage at a resolution of 7.5 nm/pixel. The volume contained 1893 horizontal 90 nm sections from the ganglion cell layer through the cone pedicles. Image registration was performed using Nornir (<http://nornir.github.io>). The transmission EM image in **Figure 4.1A** was taken of the inferior retina volume prior to sectioning.

Tissue Preparation. Retinal tissue was obtained from a terminally anesthetized male macaque (*Macaca nemestrina*) monkey through the Tissue Distribution Program at the Washington National Primate Center. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Washington. A block of inferior parafoveal retinal tissue at ~ 1 mm eccentricity from the fovea center was processed as previously described⁹². Briefly, the eyecup was placed in 4% glutaraldehyde in 0.1M sodium cacodylate buffer pH 7.4 and while in this solution a 1 mm square of retina centered 2 mm temporal to the center of the fovea was cut out and then fixed overnight at 4C. The tissue was washed in 5x5 minutes in 0.1M cacodylate buffer, then post fixed in osmium ferrocyanide for 1 hour on ice. The tissue was next washed 5x5 minutes in double distilled (dd)H₂O at room temperature (RT) and incubated in a 1% thiocarbohydrazide solution for 20 minutes at RT. The tissue was washed 5x5 minutes in ddH₂O and placed in 2% osmium tetroxide for 30 minutes at RT. The tissue was next washed in ddH₂O and *en bloc* stained in 1% uranyl acetate (aqueous overnight in the refrigerator. The next day the tissue was washed 5x5 minutes in ddH₂O, the *en bloc* stained in Walton's lead aspartate for 30 minutes at 60C. The tissue was next washed 5x5 minutes in ddH₂O and dehydrated in ice cold 30%, 50%, 70% and 95% ETOH, the allowed to come to RT. This was followed by 2 changes of 100% ETOH and two changes of propylene oxide. The tissue was then infiltrated in a 1:1 mixture of propylene oxide:Durcupan resin, for two hours and

then infiltrated overnight in fresh Durcupan. The next day, the tissue was given a fresh change of Durcupan for two hours and then placed in flat embedding molds and polymerized in a 60 C oven for two days. The block was then trimmed to approximately 0.5 mm². At this eccentricity (the edge of the foveal slope), the displacement of RGCs from cone pedicles was minimized while still remaining in a region where most midset RGCs receive single cone input.

Annotation. The serial EM volumes were annotated using the web-based, multiuser Viking software described previously²⁹ (<http://connectomes.utah.edu>). Briefly, processes were traced through the sections by placing a circular disc at the structure’s center of mass and linking the disc to annotations on neighboring sections. Cone pedicles were outlined using a closed curve polygon defined by three or more control points. Synapses were annotated with lines connected by 2–3 control points and linked to a parent neuron. Synapse identification used previously described parameters^{358,105}.

Data analysis and visualization. Data analysis and 3D rendering were performed using an open-source Matlab program (<https://github.com/neitzlab/sbfsem-tools>)²⁹. The cone pedicle analyses were based on XYZ coordinates of the closed curve control points, connected by Catmull-Rom splines. All other analysis was performed using the X, Y, Z coordinates and radius of the Disc annotations. For **Figure 4.1D**, the closed curve coordinates were used to build a volume from which isosurfaces were extracted using the marching cubes algorithm and rendered as a triangle mesh²³³. All other 3D models are triangle meshes built by rendering segments of connected annotations as rotated cylinders centered at each annotations’ XYZ coordinates and scaled by their radii.

Soma diameter was calculated from the single largest annotation in each neuron, assumed to be the soma. Primary dendrite diameter was calculated as the median diameter of annotations centered 0.5–1.5 μ m from the soma.

The probability that the reported distribution of ribbon synapses in **Figure 4.3G** was drawn by chance from a single normally-distributed group of L/M-cone OFF midset bipolar cell ribbon synapses was determined using a bootstrapping procedure. A normal distribution with the mean and standard deviation (SD) of both groups combined formed the null hypothesis. Seven integers were drawn from this normal distribution, then divided into two groups above and below the mean. The

average SD of these two groups provided a metric for the degree of bimodality – two groups drawn from a normal distribution should have large SDs while two groups from two distinct distributions should have smaller SDs, as demonstrated by the clustering in **Figure 4.3G**. The percentage of 10,000 bootstrap ribbon synapse distributions with equal or higher average SDs than the original dataset determined the reported p-value. All other reported statistics used the Mann-Whitney-Wilcoxon ranked sum. The code and data used to generate the figures in this study will be made available on GitHub upon publication.

4.5.2 Electrophysiology

Tissue Preparation. Retinal tissue was obtained from terminally anesthetized macaque monkeys (*M. nemestrina*, *M. fascicularis*, *M. mulatta* of both sexes) through the Tissue Distribution Program at the Washington National Primate Center. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Washington. Dissections were performed as previously described⁷⁵. Briefly, enucleated eyes were hemisected and the vitreous humor was removed mechanically. When necessary, the eye cup was treated for ~15 minutes with human plasmin ($\sim 50 \mu\text{g mL}^{-1}$, Sigma or Haematologic Technologies) to aid vitreous removal.

Recording. A piece of macaque macular retina with well-attached retinal pigment epithelium was placed on the stage of a microscope ganglion cell side up. The tissue was superfused with warmed (32–35 C) Ames’ medium (Sigma) at $\sim 6\text{--}8 \text{ mL min}^{-1}$. In some cases, additional D-glucose (14 mmol) was added to the Ames’ medium²³⁷. Ganglion cell spikes were measured with extracellular or loose-patch recordings using an Ames-filled borosilicate pipette. The data was sampled at 10 kHz (Multiclamp 700B, Molecular Devices), Bessel filtered at 3 kHz and digitized using an ITC-18 analog-digital board (HEKA Instruments).

Visual Stimuli. Stimulus presentation and data acquisition used the open source programs Stage (<https://stage-vss.github.io>) and Symphony (<https://symphony-das.github.io>), respectively. The Symphony stimulus protocols used in this study can be found at <https://github.com/sarastokes/sara-package>. Visual stimuli were projected onto the cone outer segments through a 10x objective (Olympus) using a Lightcrafter DLP 4500 (Texas Instruments) with a 60 Hz frame

rate. To optimize S-cone isolation, the built-in LEDs were replaced with custom LEDs at 405, 535 and 630 nm. The 405 nm primary produces relative quantal catches in S-, M-, and L-cones of 1.0, 0.20 and 0.21 respectively and thus, when used alone is about 80% S-cone isolating. This offers advantages in generating S-cone isolating stimuli over the standard 456 nm blue primary in the Lightcrafter DLP 4500 (Texas Instruments) which drives L- and M-cones more strongly with relative quantal catches of S-, M-, and L-cones of 1.0, 0.57 and 0.35 respectively.

Each LED was calibrated by measuring the spectral distribution with a spectroradiometer (Konica Minolta CS-2000) and the power with an optometer (UDT-300). A transformation matrix, A , relating the LED weights to the cone quantal catches was obtained by taking the outer product of the LED spectra ($R(\lambda)$, $G(\lambda)$ and $B(\lambda)$, as only three of the four LEDs were used at a time) and the L-, M- and S-cone spectral sensitivities ($L(\lambda)$, $M(\lambda)$, $S(\lambda)$ respectively):

$$A = \begin{bmatrix} L_R & M_R & S_R \\ L_G & M_G & S_G \\ L_B & M_B & S_B \end{bmatrix} = \begin{bmatrix} L(\lambda) & M(\lambda) & S(\lambda) \end{bmatrix} * \begin{bmatrix} R(\lambda) \\ G(\lambda) \\ B(\lambda) \end{bmatrix} \quad (4.1)$$

The transformation matrix was used to solve for the appropriate LED weights for any given level of L-, M- and S-cone activations¹¹⁴.

The mean light levels were calculated using a collecting area of $0.37 \mu\text{m}^2$ and $1 \mu\text{m}^2$ ³²⁹. All stimuli used photopic light levels ($\sim 3 \times 10^3$ to $3 \times 10^5 \text{ R}^*$). To maintain a constant state of light adaptation, the mean light level was displayed continuously between stimulus presentations. Contrast is expressed as Weber contrast.

Cell Identification and Selection. RGCs were initially identified by soma appearance, as visualized with a 60x objective (Olympus) under infrared illumination. RGC type was further determined by responses to spots, cone-isolating stimuli and mapping the receptive field with horizontal and vertical bars. Midget RGCs make up over 90% of all RGCs in the central retina³⁷⁴ and were confirmed by small soma, sustained responses and small center-surround receptive fields^{389,76}. OFF RGC somas were generally vitread to ON RGC somas. In addition to these criteria, S-OFF midget RGCs were identified using small S-cone isolating stimuli positioned over the receptive field center.

Recording Protocol. Tissue sensitivity was assessed at the beginning of each experiment by ensuring ON parasol RGCs responded to a full-field ($\sim 1\text{mm}$), 5% contrast, 4 Hz temporally-modulated spot³⁶⁰. Parasol RGCs lack significant S-cone input and were also used to validate the S-cone isolating stimuli (**Figure 4.4A**)¹²¹.

For each subsequent RGC encountered, the polarity (ON, OFF or ON-OFF) and cell type were first determined by spots presenting high contrast luminance increments and decrements from a photopic mean light level. Cell type was confirmed by receptive field dimensions. The receptive field center was determined by vertical and horizontal bars, presented as 2–4 Hz squarewave temporal modulations. The center and surround receptive field radii were measured from Difference of Gaussian fits to expanding spots and annuli. In cases where the receptive field dimensions were unclear (either due to noise or a rare cell with an atypical receptive field), the measurements were confirmed by estimating the spatiotemporal receptive field using coarse (25–50 μm square pixels) binary spatial noise.

S-cone contributions were measured in each RGC with different sizes of S-cone isolating spots, presented as 1–2 Hz squarewave temporal modulations. For each RGC with a significant S-cone response, the temporal and spatial characteristics were next measured with S-cone isolating full-field temporally modulated Gaussian noise and drifting gratings, respectively.

Expanding spot: High contrast luminance spot stimuli were positioned at the receptive field center and presented as a 4 Hz temporal modulation.

Gaussian noise: The temporal “white noise” stimulus was generated by psuedo-random draws from a Gaussian distribution centered at the mean light level (mean = 50%, SD = 30% contrast). The noise stimulus was presented in 10 or 20 second epochs, with 1 second interval between epochs.

Analysis. To extract the spike responses, recorded currents were high-pass filtered, then sorted using k-means clustering. In some cases, the returned spikes were further sorted by setting a manual amplitude threshold. Analysis of the expanding spot stimuli were based on the F1 amplitude, the response at the temporal modulation frequency. The F1 amplitudes were calculated from the cycle-averaged spike rate, binned at 60 Hz. Unless otherwise specified, data are expressed as mean $\pm$ SEM. Significance was determined using the Mann-Whitney-Wilcoxon rank sum test. All analyses were performed in Matlab 2017b (Mathworks) and the final figures prepared in Igor Pro 7 (Wavemetrics,

Oswego WA).

Difference of Gaussians model. A Difference of Gaussians (DoG) model was fit to the F1 amplitudes^{310,113}. The DoG model characterizes the center and surround receptive fields as two antagonistic, two-dimensional Gaussians with separate strengths and sizes. The center and surround receptive fields are assumed to be radially symmetric and centered at the same location. The DoG model predicts the response, R , for spot diameter, f , as:

$$R(f) = R_0 + (K_c \pi \sigma_c^2 e^{-(\pi \sigma_c f)^2}) - (K_s \pi \sigma_s^2 e^{-(\pi \sigma_s f)^2}) \quad (4.2)$$

where R_0 is the baseline response, σ_c and σ_s are the center and surround receptive field sizes, respectively, and k_c and k_s are the center and surround strengths, respectively.

White noise analysis. Analysis was performed on the spike rate, binned at 360 Hz. The first second of the response was omitted to control for adaptation. The linear filter, F , is obtained by cross-correlating the stimulus, $s(t)$, with the spike rate, $r(t)$, and dividing out the stimulus' power spectrum:

$$\tilde{F}(\omega) = \frac{\tilde{s}^*(\omega) \tilde{r}(\omega)}{\tilde{s}^*(\omega) \tilde{s}(\omega)} \quad (4.3)$$

where $\tilde{s}(\omega)$ is the Fourier transform of $s(t)$, $\tilde{r}(\omega)$ is the Fourier transform of $r(t)$ and $*$ denotes the complex conjugate. In practice, the stimulus power spectrum was nearly flat and the denominator was omitted³⁶⁹. The inverse transform of $\tilde{F}(\omega)$ returned the time-domain linear filter, F .

CHAPTER 5

The Spectral Sensitivity of the Neurons Mediating Black and White

The study presented in this chapter has been submitted and is available as a preprint:

Patterson, S.S., Neitz, M., Neitz, J (2020) The Spectral Sensitivity of the Neurons Mediating Black and White. bioRxiv

5.1 Summary

Black and white sensations and their contributions to color saturation are not equally strong across the visual system, but instead have a spectral tuning defined by the ways in which their neural substrates process the outputs of three univariant cone photoreceptors. The neurons mediating black and white and how they combine the cone outputs remain controversial; however, growing evidence indicates cone-opponent midgen ganglion cells are involved. Here we investigate whether midgen ganglion cells can account for the variation in perceived saturation with wavelength, as measured by the classic saturation discrimination experiments. The results are traditionally explained by models assuming specific psychophysical functions represent black, white and hue sensations.

5.2 Introduction

Our experience of color can be defined by three parameters: hue, brightness and saturation. A fundamental question in color vision research is how these perceptual features relate to the physical attributes of incoming light and how they are represented by neurons within the visual system. Of these three parameters, saturation has been the most difficult to link to an underlying neural

substrate.

Saturation is the perceived distance of a color from white, gray or black (black-white)³³⁴. For example, a patch of monochromatic blue light against a relatively dark background appears very saturated, producing little or no white sensation. Between unique blue and equal energy white, the perceived color becomes increasingly desaturated as the strength of the white sensation increases relative to the blue hue sensation. The same applies to the perceived distance from black for colored patches against lighter backgrounds – navy appears desaturated because the black sensation is strong relative to the blue hue sensation. When matched for brightness, colors have two components - hue and black-white - with the relative strengths of these two components defining the perceived saturation^{244,159}.

The relative amounts of hue and black-white perceived in monochromatic lights vary reliably as a function of wavelength. Trichromats universally agree that of the four fundamental hues (red, green, blue and yellow), yellow is the most desaturated (i.e. contains the most white) and blue is the most saturated. Red is perceived as slightly less saturated than blue while green is perceived as slightly more saturated than yellow. This phenomenon is quantified in classic saturation discrimination experiments, such as **Figure 5.1A** and **Figure 5.1D**. The resulting saturation discrimination function is interpreted as reflecting the relative activity of the neural substrates for hue and black-white as a function of wavelength. However, the identities of the neural substrates for both hue and black-white remain highly controversial.

Classic models fitting the saturation discrimination function assumed black-white was mediated by neurons summing L- and M-cones ($L + M$)^{177,148,171,90}. However, there is now growing evidence that our percepts of black and white are instead mediated by spectrally-opponent ($L - M$) midget ganglion cells^{89,284,44,318}. The purpose of this study is to determine whether the spectral sensitivities of midget ganglion cells can account for the saturation discrimination function.

5.3 Methods

5.3.1 Saturation discrimination experiments

There are several different experimental paradigms for measuring saturation as a function of wavelength and they produce different results (**Figure 5.1A**, **5.1B**, **5.1D**). Though undoubtedly related to saturation, the results of some paradigms do not perfectly match our perceptual experience of

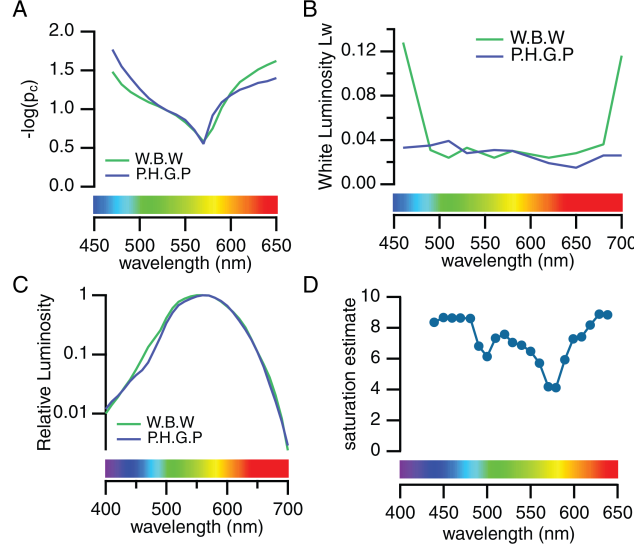


Figure 5.1 (A) Saturation discrimination curves measured as the "first step from white" from Wright & Pitt (1937)³⁹³. (B) Saturation discrimination curves measured as the "first step from the spectrum" from Wright & Pitt (1935)³⁹⁴. (C) Spectral luminosity functions, $V(\lambda)$, from the same two observers, obtained from Wright & Pitt (1935)³⁹⁴. (D) Saturation scaling experiment from Jacobs (1967)¹⁷².

saturation²⁹⁸. Though the paradigms are sometimes grouped together for analysis and interpretation¹⁷⁷, here we will examine each paradigm individually.

First Step from White Paradigm. Our primary focus will be modeling a classic experimental paradigm for quantifying the wavelength dependence of saturation that measures the “first step from white”³⁹⁷ or the “chromatic threshold with respect to white”²⁹⁸ (**Figure 5.1A**). A broadband light (perceived as white) is presented to the entire field while a monochromatic spectral light is added to just one half of the field (**Figure 5.4A**). The strength of the spectral light is increased until just noticeably different from the broadband light. In other words, until a just noticeable difference in saturation occurs. Importantly, the two lights are also matched for brightness. Then the least perceptible colorimetric purity (p_c) can be obtained by:

$$p_c = \frac{L_\lambda}{L_w + L_\lambda} \quad (5.1)$$

where L_λ is the intensity of the spectral light and L_w is the intensity of the broadband light. This metric expresses saturation independent of both the intensity of the broadband light and the wavelength of the spectral light²⁹⁸. By convention, the results are plotted as the inverse log,

$-\log(p_c)$, so that the y-axis reads out as a measure of saturation as a function of wavelength. In other words, **Figure 5.1A** can be read as saying the participant had to increase the intensity of the 570 nm light far more than the intensity of the 470 nm light to produce a discriminable change in saturation, defined as a just-noticeable-difference in hue.

First Step from the Spectrum Paradigm. The “first step from the spectrum” experiments in **Figure 5.1B** measure the minimal amount of a broadband light that must be added to a monochromatic spectral light to produce a just-noticeable decrease in saturation. While initial studies found relatively flat sensitivity across the visual spectrum^{298,393}, subsequent work reported more wavelength-dependence¹⁸⁴ and an interesting relationship with mean light level not seen in the “first step from white” paradigm³⁹⁷. Additional studies will be necessary to address these inconsistencies so we do not attempt to fit these results here.

An important point is that both the “first step from white” and the “first step from the spectrum” rely on threshold changes in saturation, yet the results are strikingly different (compare **Figure 5.1A** to **Figure 5.1B**). However, the way each experiment defines the saturation threshold is also different. The “first step from white” measures a threshold hue percept while the “first step from the spectrum” measures a threshold white percept.

Saturation Scaling Paradigm. The saturation scaling paradigm in **Figure 5.1D** asks participants to rate the saturation of equiluminant spectral lights on a fixed scale^{127,172,139}. Saturation scaling is distinct from the “first step from white” and “first step from the spectrum” paradigms because it is not a threshold measurement but rather a direct judgment of perceived saturation.

5.3.2 Model

Cone-opponent midget ganglion cells. Traditionally, accounts of the physiological basis of saturation have assumed contributions from spectrally-opponent neurons for hue and spectrally non-opponent neurons for black-white⁸⁷ (but see De Valois & Marrocco, 1973⁹¹). Here we took a different approach and limited our model to the midget ganglion cell subtypes proposed to be involved in black and white (**Figure 5.2**). Each L- and M-cone photoreceptor in the central retina is represented twice in the retinal output, by both an ON and OFF midget ganglion cell¹⁹⁵. In

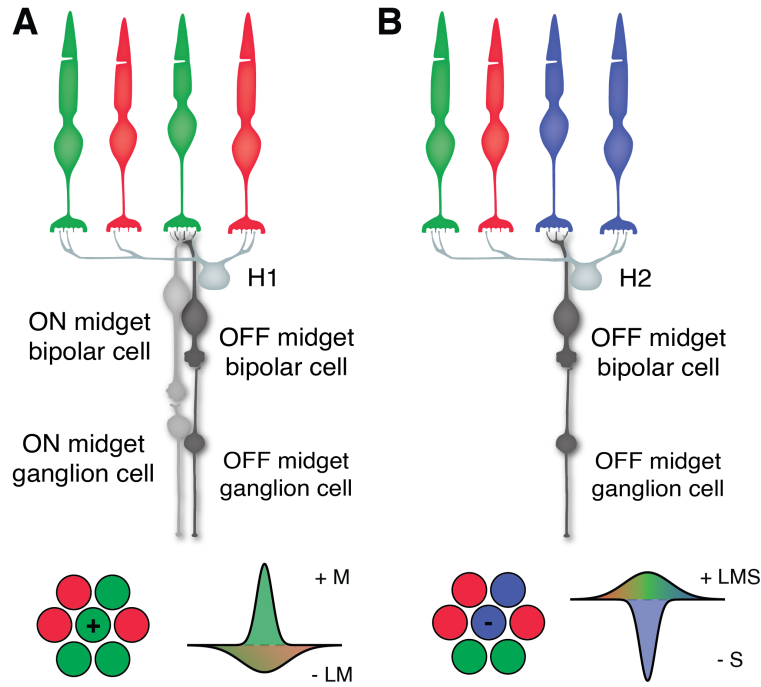


Figure 5.2 Spatial and spectral receptive fields of midget ganglion cell subtypes. **(A)** Circuitry for M-ON and M-OFF midget ganglion cells. The circuitry for L-ON and L-OFF midget ganglion cells is identical, but with an L-cone directly contacting the midget bipolar cells and forming the center receptive field (not pictured). **(B)** Circuitry for S-OFF midget ganglion cells. There is no S-ON midget ganglion cell. The photon catch in the center cone is compared to the photon catch in surrounding cones provided by H1 or H2 horizontal cell feedback^{366,278?}. Bottom: Illustration of the cone inputs and sensitivity profiles for M-ON and S-OFF midget ganglion cell receptive fields. The polarity of the center cone is marked with '+' or '-' for ON and OFF midget ganglion cells, respectively.

addition, a controversy regarding S-cone midget ganglion cells^{189,221} was recently resolved and it was confirmed that each S-cone has an OFF midget ganglion cell, but not an ON midget ganglion cell²⁸². Midget ganglion cells have center-surround receptive fields that provide both spatial and spectral opponency (**Figure 5.2**, reviewed in Patterson et al., 2019²⁸⁴).

The outputs of midget ganglion cell center-surround receptive fields are a measure of the relative photon catch in the center and surround receptive fields. Their outputs vary as a function of wavelength because the cone inputs to the center and surround receptive fields differ and each cone's response is univariant, confounding changes in wavelength with changes in intensity^{316,19}. Here, we modeled the wavelength-dependence of the midget ganglion cell's output as the difference

between the spectral sensitivities of the center and surround receptive fields (**Figure 5.3**)^{324,326}.

$$L_{on}(\lambda) = \alpha * L(\lambda) - (1 - \alpha) * M(\lambda) \quad (5.2)$$

$$M_{on}(\lambda) = \beta * M(\lambda) - (1 - \beta) * L(\lambda) \quad (5.3)$$

$$M_{off}(\lambda) = (1 - \gamma) * L(\lambda) - \gamma * M(\lambda) \quad (5.4)$$

$$L_{off}(\lambda) = (1 - \delta) * M(\lambda) - \delta * L(\lambda) \quad (5.5)$$

$$S_{off}(\lambda) = (1 - \epsilon) * (L(\lambda) + M(\lambda))/2 - \epsilon * S(\lambda) \quad (5.6)$$

where $L(\lambda)$, $M(\lambda)$ and $S(\lambda)$ represent the L-, M- and S-cone spectral sensitivities, respectively, corrected for optical density and macular pigment. The Neitz photopigment template was used, assuming peak sensitivities of 559, 530 and 419 nm and optical densities of 0.3, 0.3 and 0.35 for the L-, M- and S-cones, respectively⁴⁹. The center-surround balance is represented by α , β , γ , δ and ϵ for M-ON, L-ON, M-OFF, L-OFF and S-OFF midget ganglion cells, respectively. The output of each model midget ganglion cell was rectified to simulate the nonlinearity imposed by the spike threshold of a ganglion cell. The equations for the five spectral subtypes of midget ganglion cells are schematized in **Figure 5.3**.

While these equations appear to maximize spectral opponency, the presence of, for example, L-cones in the surround of the L-OFF midget ganglion cell, can be accounted for by decreasing the α parameter. This parameter can be varied to account for the spatial structure of the stimulus (discussed in the **Results**). This term also reduces the number of assumptions we need to make about crucial unknown variables, such as the relative strength of the center and surround receptive fields and the participant's L:M-cone ratio.

Saturation models. Classic saturation models proposed color saturation was mediated by the ratio of activity in the neural substrates for hue and black-white^{177,171,148,87}. The spectral sensitivities of the neural substrates for hue were assumed to match the red-green and blue-yellow opponent response functions obtained with hue cancellation^{170,381}, while the neural substrates for black-white were assumed to match the spectral luminosity function, $V(\lambda)$, obtained with heterochromatic flicker^{58,378}. Our approach is distinct as we instead constrained our model to the features of midget ganglion cell receptive fields established through anatomical and physiological

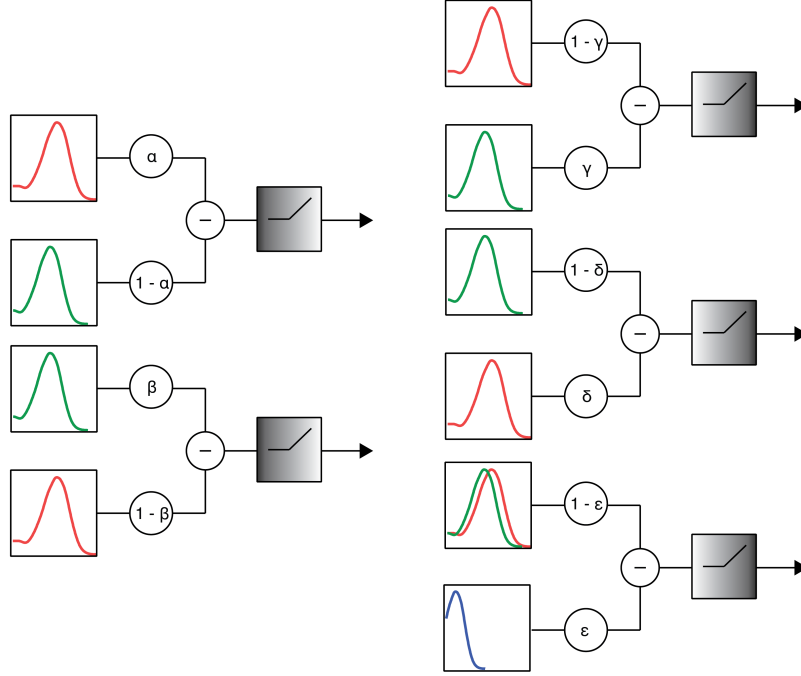


Figure 5.3 The output of a midjet ganglion cell is a measure of relative photon catch in the center and surround receptive fields. However, this varies as a function of wavelength because the center and surround receptive fields have different spectral sensitivities. To capture this, we modeled the outputs of the five spectral subtypes of midjet ganglion cell as a function of wavelength. L-ON and M-ON midjet ganglion cells are on the right. L-OFF, M-OFF and S-OFF midjet ganglion cells are on the left. Cone spectral sensitivities for the center and surround receptive fields are weighted with opponent gain parameters, subtracted from each other then passed through a rectifying nonlinearity. The final saturation discrimination models combine these five independent retinal output pathways in various ways to account for the "first step from white" saturation discrimination function.

experiments^{282,366,278,195,189}. The resulting midjet ganglion cell spectral sensitivities are distinct from the opponent response functions and the spectral luminosity function obtained through psychophysics experiments. Because the output of each midjet ganglion cell subtype is rectified, the models developed in the **Results** ((5.7) and (5.8)) are linear combinations of the spectral sensitivities of the outputs of midjet ganglion cells, not the L-, M- and S-cone spectral sensitivities, as in other color vision models that account for the saturation discrimination function^{177,171,148}.

We adopted three standard assumptions common to models fitting the saturation discrimination function. First, that there is a set of neurons whose activity directly determines the strength of our fundamental percepts of red, green, blue, yellow, black and white. We refer to these as the neural substrates of hue and black-white. A second assumption is that ON cells mediate percepts of white while OFF cells mediate percepts of black^{89,363,382}. Third, we assume that the characteristics of the underlying neural substrates of hue and black-white (in our case, the midjet ganglion cells) can

be combined linearly to account for the saturation discrimination function. Linear combinations are generally considered valid for experiments measuring threshold discriminations¹⁴⁸, such as the "first step from white" paradigm.

Model Fitting Procedure. We obtained saturation discrimination data for the "first step from white" paradigm from published tables^{298,244,393,119}. The scaling data was extracted from a published figure¹⁷². The models were fit to each individual saturation discrimination curve dataset in MATLAB (Mathworks) using the `lsqcurvefit` function. The y-axis scaling varied between experiments, so a scaling parameter, θ , was included. The resulting curves were translated along the y-axis to align with the minimum of each saturation discrimination dataset. Additional details about each model are discussed as they are introduced in the **Results**.

5.4 Results

5.4.1 Development of a Midget Ganglion Cell Model for Saturation Discrimination

We began by asking whether midget ganglion cells alone could account for the results of the "first step from white" paradigm, as this is the classic method for obtaining saturation discrimination functions. As described in **5.3.1**, the participant increases the intensity of a monochromatic spectral light until a just-noticeable-difference in hue is observed, relative to the matching field, which is illuminated with a broadband light that is perceived as white.

Model 1: LM-ON midget ganglion cells. Because the experiment directly involved the perceptual experience of white, we began by asking whether ON midget ganglion cells could account for the saturation discrimination function. While it was clear their spectral sensitivities could not provide a full account, we asked whether they could at least explain a particularly salient feature: the sharp minimum around 570 nm.

We modeled the spectral sensitivity of L-ON and M-ON midget ganglion cells using (5.2) and (5.3), respectively. When the center and surround receptive fields were equally weighted ($\alpha = \beta = 0.5$), the peak in white percepts at 570 nm was not predictable from the spectral sensitivities of the ON midget ganglion cells (**Figure 5.4C**). Once rectified to account for the midget ganglion cell's spike threshold, the summed L-ON and M-ON spectral sensitivities do not peak near 570 nm.

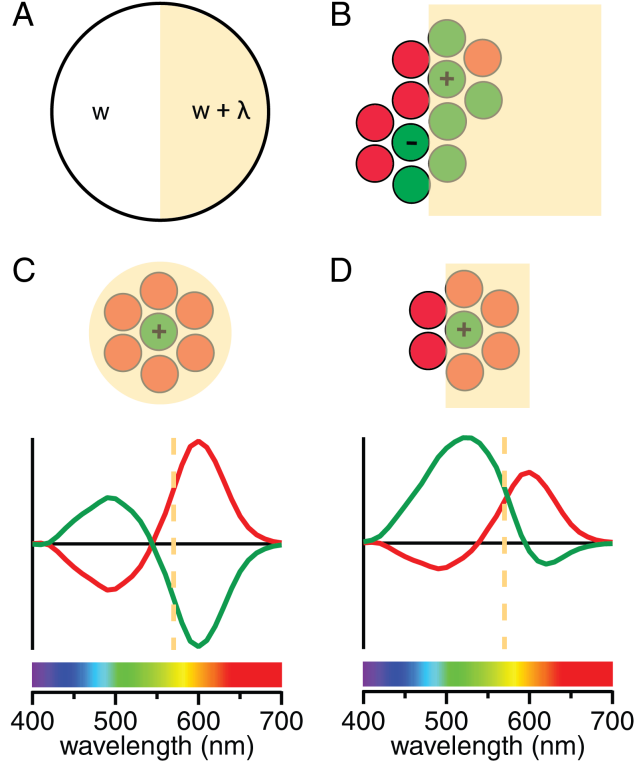


Figure 5.4 (A) Example stimulus for a saturation discrimination stimulus experiment. (B) Cone inputs to L/M midget ganglion cells at the edge of the test light. An ON-center cell responds best at the inner edge of the test light while an OFF-center cell responds best outside the test stimulus. Note that the model also includes a midget ganglion cell of the opposite polarity at each center cone. (C, D) Top: Illustration of full-field and edge stimuli superimposed on the receptive field of a sample M-ON midget ganglion cell. Bottom: Corresponding L-ON and M-ON midget ganglion cell spectral sensitivities before rectification. Yellow dashed line at 570 nm.

The approach in **Figure 5.4C** considers only the spectral tuning of the cone inputs to midget ganglion cells and does not account for the spatial arrangement of those cone inputs. The combination of spectral and spatial opponency in midget ganglion cells means wavelength and spatial structure are confounded. **Figure 5.4C** overlooks the midget ganglion cell’s spatial opponency and essentially isolates the spectral opponency by modeling the spectral response of a neuron to a spatially-homogeneous monochromatic stimulus. Yet there is considerable evidence that our perception of hue and black-white is instead defined by the changes in intensity and wavelength that occur at borders^{209,144}. The saturation of the spectral light is likely defined by the receptive fields of neurons located along the edge of the stimulus and the saturation of the interior is set by “filling in” at the level of the cortex. Consistent with this idea, the center-surround receptive fields of midget ganglion cells are ideal edge detectors and encode spatial contrast, such as that occurring at

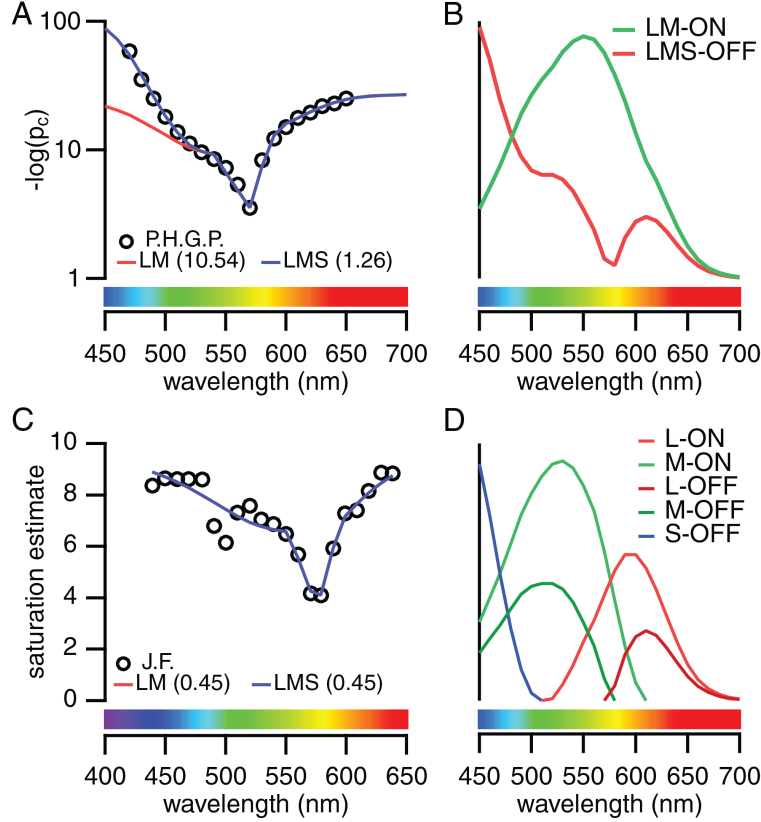


Figure 5.5 (A) Midget ganglion cell models with (blue curve) and without (red curve) the S OFF midget ganglion cell fit to the saturation discrimination data for one observer, P.H.G.P., from Wright & Pitt (1937)³⁹³. The root mean square error is shown in parentheses. (B) Summed spectral sensitivities for ON and OFF midget ganglion cell subtypes for the fit in A. (C) Models fit to saturation scaling data for one observed, J.F., from Jacobs (1967)¹⁷². (D) Spectral sensitivities for each spectral subtype of midget ganglion cell for the fit in A.

a border^{242,284}. As shown in **Figure 5.4D**, if the receptive field is located at an edge and only some of the surround is stimulated (or the weight parameters α and β are adjusted), the contributions of both types now could sum to signal white at 570 nm. Because spatial and spectral information are confounded in the midget ganglion cell's output, changes in the spatial structure of the stimulus will alter the midget ganglion cell's spectral sensitivity.

Model 2: LM-ON and LM-OFF midget ganglion cells. The L-ON and M-ON midget ganglion cell spectral sensitivities in **Figure 5.4D** can contribute to peak white sensations at 570 nm but cannot account for the steep increases in saturation surrounding 570 nm. In other words, the strength of white percepts in the middle wavelengths is too broad and would need to be attenuated to fully account for the saturation discrimination function. We next investigated

whether including OFF midget ganglion cells could provide a full account by asking whether the saturation discrimination function could be fit by a model combining the outputs of LM-ON and LM-OFF midget ganglion cells (but without S-OFF midget ganglion cell contribution).

$$Y(\lambda) = \theta * (L_{on} + M_{on} - \phi * (L_{off} + M_{off})) \quad (5.7)$$

where ϕ represents the gain of the OFF midget ganglion cells and θ is the scaling parameter introduced in the **Methods**. Note that opponency between the ON and OFF cells is not strictly enforced by the model as in (5.2)-(5.6). The OFF gain parameter, ϕ , only determines the strength of the OFF cells relative to the ON cells, which were set to an arbitrary gain of 1.

The results (red curve in **Figure 5.5A**) demonstrate excellent fits to the middle and long wavelength regions of the saturation discrimination function. L vs. M cone-opponency in midget ganglion cells provides a clear explanation for the sharp minimum at 570nm. However, the model fails to account for the slope of increasing saturation for short-wavelength lights and instead predicts monochromatic blues and reds would be equally saturated.

Model 3: LM-ON and LMS-OFF midget ganglion cells. Accounting for the short-wavelength component of the saturation discrimination function appears to require contribution from S-cones. There has been considerable controversy regarding the existence of S-OFF midget ganglion cells and these neurons have not been incorporated into models of visual perception. We recently provided both anatomical and physiological confirmation of the existence of S-OFF midget ganglion cells in the primate central retina²⁸². . Briefly, we confirmed a single OFF midget bipolar cell contacted each S-cone, which in turn, contacted a single OFF midget ganglion cell. We also confirmed that, like the more common L vs. M-cone midget ganglion cells, S-OFF midglets have a center-surround receptive field consistent with a role in spatial vision.

To determine whether the S-OFF midget ganglion cells are necessary to account for the saturation discrimination function, we added an S-OFF term ((5.6)) to the previous model.

$$Y(\lambda) = \theta * (L_{on} + M_{on} - \phi * (L_{off} + M_{off}) - \omega * S_{off}) \quad (5.8)$$

The S-OFF term was given a separate gain control (ω) from the L/M-OFF terms to allow the

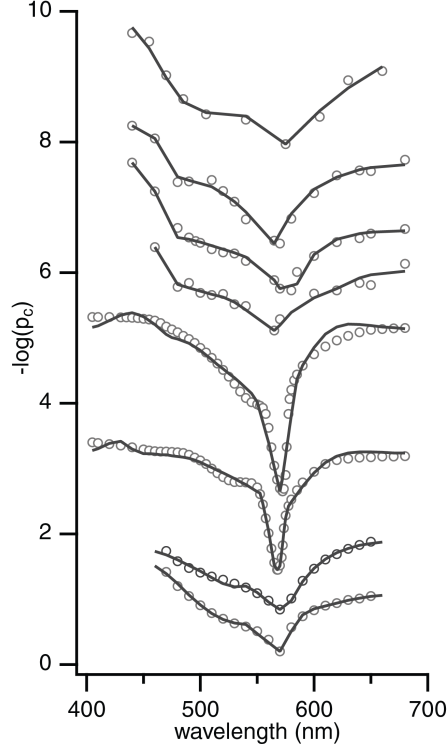


Figure 5.6 Model fits (grey) to classic saturation discrimination datasets (black circles). From top to bottom: D.P. from Purdy (1931)²⁹⁸, W.J.M., L.C.M. and F.L.W., from Martin et al (1933)²⁴⁴, I.G.P. and F.G.B. from Priest & Brickwedde (1938)²⁹³, W.D.W. and P.H.G.P. from Wright & Pitt (1937)³⁹³.

model to account for the sparsity of S-cones and the potential for their signals to be amplified downstream^{312,88}.

Including the S-OFF midget ganglion cell allowed our model to fit the short-wavelength region of the saturation discrimination function (blue curve in **Figure 5.5A**). To provide intuition on how the model worked, the spectral sensitivities of the outputs of each midget ganglion cell subtype are shown in **Figure 5.5D** and the ON and OFF subtypes summed together are shown in **Figure 5.5B**. Our model provided excellent fits to many "first step from white" datasets (**Figure 5.6**)^{298,293,393,244}.

5.4.2 Application of Model to the Saturation Scaling Paradigm

We next asked whether our models could account for the results of the saturation scaling paradigm. Some past models have grouped scaling and "first step from white" saturation discrimination functions together as being roughly similar¹⁷⁷. However, there are several reliable differences between results obtained by the two paradigms (compare **Figure 5.1A** and **Figure 5.1D**). The clearest

difference is the strong decrease in saturation at 490-500 nm obtained with the scaling paradigm and the steeper short-wavelength slope obtained with the "first step from white" paradigm.

Attempting to fit the results of the saturation scaling paradigm with our models was not as successful but revealed two interesting features (**Figure 5.5C**). First, our model cannot account for the dip in saturation at 490-500 nm or the shallow slope in the short wavelength region. Second, the S-OFF midget ganglion cell parameter did not alter the fit, indicating this parameter was necessary for the "first step from white" paradigm, but not for the scaling paradigm.

5.4.3 Saturation Discrimination in Dichromats

Our model demonstrated that the L vs. M cone opponency in midget ganglion cells can explain the sharp minimum in saturation around 570 nm. Additional support for this conclusion can be seen in the saturation discrimination functions of dichromats (**Figure 5.7A**)^{119,270,59,392}. The effect of a missing L-, M- or S-cone on the spectral luminosity function, $V(\lambda)$ is predictable - the peak shifts towards the remaining long-wavelength cone types (**Figure 5.7B** and **Figure 5.7D**). However, the effect of dichromacy is more complex for saturation discrimination functions. The sharp decrease in saturation around 570 nm is absent for protanopes and deuteranopes (**Figure 5.7A**), but remains desaturated for tritanopes (**Figure 5.7C**)⁵⁹. Interestingly, for deuteranopes and protanopes, the saturation discrimination functions are closer to matching our intuition of the ideal white (i.e. equal sensitivity at all wavelengths) than the spectral luminosity functions (**Figure 5.7A**).

5.5 Conclusions

We find that spectrally-opponent midget ganglion cells can account for the saturation discrimination function measured using the "first step from white" paradigm. The midget ganglion cell models that successfully fit the "first step from white" paradigm could account for some, but not all, of the features of the scaling paradigm. Surprisingly, an S-OFF midget ganglion cell contribution was required to fully explain the "first step from white" saturation discrimination function. This is the first quantitative model to propose a role for the S-OFF midget ganglion cell in visual perception. Though the density of S-cones and S-OFF midget ganglion cells is far lower than L/M-cones and L/M-OFF midget ganglion cells, their contribution may be stronger because there is no opposing response from S-ON midget ganglion cells. An interesting avenue for future research will be to ex-

plore whether the S-OFF midget ganglion cells can explain any of the well-documented asymmetries in the S-cone ON and OFF pathways.

5.5.1 *Black and white percepts vs. the neural substrates for black and white*

The saturation discrimination experiment does not directly involve sensations of black, however, the need for OFF midget ganglion cells to account for the “first step from white” paradigm suggests that the spectral sensitivity of white sensations is shaped by the neural substrates for both white and black. Indeed, some form of opponency is inherent in the anatomy and physiology of the ON and OFF pathways (reviewed by Westheimer, 2007³⁸²) and opponency between black and white, is assumed in standard saturation models^{177,148,171}. Importantly, our interpretation is meant to account for the saturation discrimination function and not for other measures of blackness and whiteness^{128,26,378}.

5.5.2 *Interpreting the saturation discrimination experiment*

The logic used to interpret the results of saturation discrimination experiments is as follows. The saturation of color depends not only on the strength of the hue component, but also the black-white component. If saturation is the relative portions of hue and black-white in a monochromatic light, then saturation experiments should measure how the relative amounts of hue and black-white vary as a function of wavelength. These variations as a function of wavelength reflect the spectral sensitivities of the neurons mediating hue and black-white sensations. It follows that saturation discrimination experiments can be interpreted as *a comparison between the spectral sensitivities of the neural substrates mediating our percepts of hue and black-white*.

Past models of saturation are based on the assumption that saturation represents the ratio of activity in the independent chromatic and achromatic channels, or the neural substrates of hue and black-white^{177,148,171}. This can be expressed as an equation, like Hurvich & Jameson’s saturation coefficient:

$$Saturation = \frac{hue}{hue + blackwhite} \quad (5.9)$$

that proposes a relationship between the neural substrates for hue and black-white in defining color

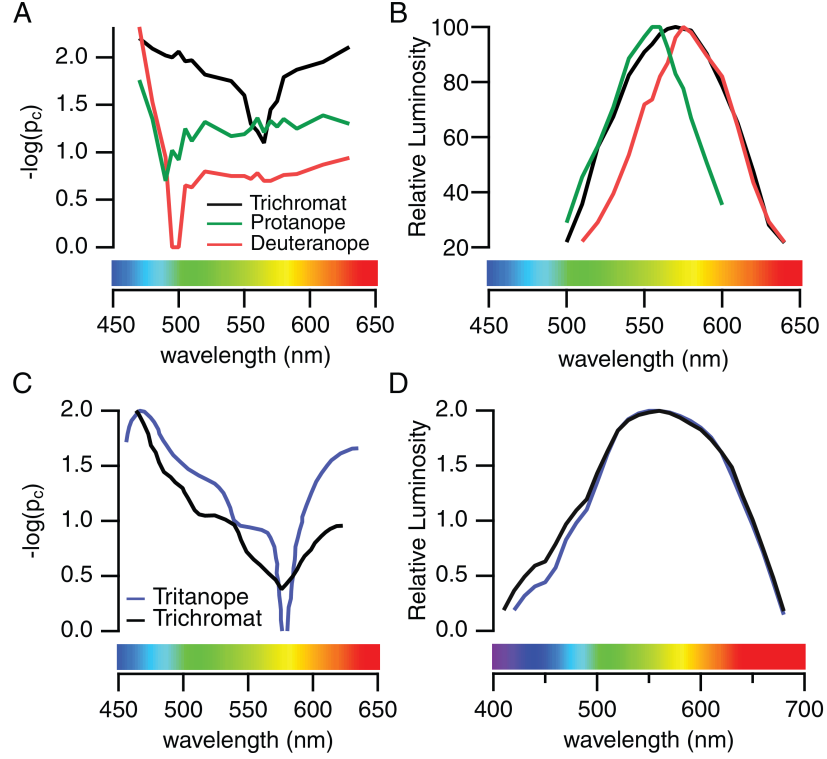


Figure 5.7 (A) Saturation discrimination and (B) spectral luminosity functions for trichromatic, deuteranopic and protanopic observers, from Chapanis (1944)¹¹⁹. (C) Saturation discrimination function for a tritanope and trichromat, from Cole (1966)⁵⁹. (D) Average spectral luminosity functions for seven tritanopes compared to a trichromatic observer obtained under the same conditions, from Wright (1952)³⁹².

saturation. Regardless of the hue response to a given light, the perceived color saturation will also depend on the extent to which the neural substrates for black-white are simultaneously responding. For any given response from the neural substrates for hue, the color perceived will be less saturated if there is a large response from the neural substrates for black-white and more saturated if the black-white component of the total response is relatively small¹⁷⁰.

At each wavelength tested in the "first step from white" paradigm, the participant adjusted the intensity of the spectral light until a just-noticeable-difference (JND) in hue was perceived. Thus, at each wavelength tested, hue percepts are constant at 1 JND, a perceptual unit. Assuming our hue percepts correlate with activity in neural substrates for hue, we can propose that, for each wavelength tested in the "first step from white" paradigm, both the strength of the hue percept and the activity in the neural substrates for hue is effectively constant at 1 JND. This makes the results of the "first step from white" paradigm in **Figure 5.5A** a function of only one variable: black-white (**Equation** (5.9)). The stronger the response from the neural substrates for black-white, the

greater the intensity of the spectral light necessary to elicit a threshold hue response. The neural substrates for black-white respond to both the broadband light and the spectral light, and their spectral sensitivity determines the strength of their response to the spectral light. Thus, the "first step from white" paradigm can be interpreted as *revealing the underlying spectral sensitivity of the neural substrates for black-white*.

To put this interpretation in context, previous saturation models focused on the “first step from white” as a threshold change in saturation. For example, Hurvich & Jameson’s model was designed to find the hue and black-white (their chromatic and achromatic channels) responses necessary to give a just-noticeable-change in saturation. They assumed saturation was effectively constant at each wavelength tested and set (5.9) to be equal to 0.1, relative to 0 for a completely desaturated light¹⁷⁸. As mentioned in the **Introduction**, when matched for brightness, each color has two components: hue and black-white and their relative strengths determine the perceived saturation. Here we propose that, in the case of the “first step from white” paradigms, the threshold change in saturation can be described more specifically as a threshold change in the hue component (i.e. a just-noticeable-difference in hue).

5.5.3 *Different saturation experiment paradigms measure distinct aspects of saturation.*

We now may be able to explain some of the variation between different measures of saturation discrimination, as shown in **Figure 5.1**. In one of the first studies of saturation discrimination, Purdy (1931) concluded the “first step from white” results are closely related to saturation, but do not completely match our perceptual experience of saturation²⁹⁸. Here, we reason they measure black-white instead.

The most accurate experiment for measuring our perceptual experience of saturation may be the results of saturation scaling experiments like **Figure 5.1D** because these tasks involved direct judgments of perceived saturation. The scaling experiments capture both the desaturation of yellows (~ 570 nm) and cyans (~ 490 nm) while **Figure 5.1A** emphasizes only the desaturation around 570 nm. We can reason that, unlike the “first step from white” experiments, scaling experiments engage both the neural substrates for hue and black-white. In other words, the results are a function of both variables in (5.9). Thus, a minimum in saturation can occur because the black-white response is relatively strong, or because the hue response is relatively weak. Comparison with opponent

response functions^{170,381} indicate the latter is the case for the 490-500 nm region.

Our approach may also be able to explain the striking differences in saturation measured as the “first step from the spectrum” (**Figure 5.1B**)^{397,184,298}. Like the “first step from white”, a threshold change in saturation is measured, however, in the “first step from the spectrum” paradigm, it is defined as a JND in white, not hue (see **Methods**). We can reason that this is a situation where black-white sensations are held constant and only hue sensations and the neural substrates of hue are predicted to vary. Thus, the “first step from the spectrum” paradigm measures a distinct process (the neural substrates of hue) and would not be expected to match the results in **Figure 5.1A** or **Figure 5.1D**.

Our model indicates that the spectral opponency provided by the midget ganglion cell’s center-surround receptive field is crucial for establishing the spectral sensitivity of downstream percepts. An interesting implication is that any experimental condition that minimizes contributions from spatial opponency will result in non-opponent spectral sensitivity. For example, spatial opponency weakens at high temporal frequencies^{333,210} and low light levels¹⁶ so experiments involving these conditions are predicted to reveal spectral sensitivities like $V(\lambda)$, the sum of L- and M-cones without evidence for spectral opponency.

5.5.4 What is “achromatic”?

As discussed above, early saturation models, such as Hurvich & Jameson’s opponent process theory, represented saturation as the ratio of activity in chromatic and achromatic channels^{177,170}. Hurvich & Jameson’s achromatic channel refers to black, white and gray, or the absence of any hue sensations. However, vision scientists often associate “achromatic” with neurons that are spectrally non-opponent, summing the responses of different cone types, and unable to distinguish changes in wavelength from changes in intensity. Meanwhile, “chromatic” neurons are spectrally-opponent and compare the activities of different cone types.

Accordingly, Hurvich & Jameson (1955) assumed the mechanisms mediating black-white percepts (their “achromatic channel”) matched $V(\lambda)$, the spectral luminosity function. Thus, “achromatic” has often been equated with luminance, $V(\lambda)$, L+M-cone activity, and later, the activity of non-spectrally-opponent neurons, particularly the parasol ganglion cells²¹³. We note in passing that there are other $L + M$ ganglion cells in the primate retina with similar response properties to

parasol ganglion cells⁶⁴ and $V(\lambda)$ can also be obtained from the responses of $L - M$ midget ganglion cells²²⁶.

Saturation has proven difficult to account for by models assuming black and white percepts are mediated by neurons with spectral sensitivities matching $V(\lambda)$. **Figure 5.1A** and **Figure 5.1C** show the saturation discrimination and luminosity functions for the same two observers^{394,393}, which diverge in two key features. If the "first step from white" experiment measures the spectral sensitivity of the neural substrates for black-white, then models using $V(\lambda)$ as a starting assumption may need to be revisited. The role of $V(\lambda)$ is well-summarized by a review on luminance by Lennie and colleagues (1993): " $V(\lambda)$ is a useful and well-established standard for photometry, but beyond that its significance for visual function is unclear"²²⁶.

5.5.5 *Spectrally-opponent midget ganglion cells can account for black-white sensations*

Increasing evidence supports the idea that midget ganglion cells mediate high resolution achromatic spatial vision (reviewed by Lennie & Movshon (2005)²²⁵). As discussed above, their role in spatial vision underlies their ability to encode black and white percepts, because these are defined at the borders of objects^{144,209,274}. The center-surround receptive fields of ON and OFF midget ganglion cells are perfectly suited for this task²⁴² - they encode increments and decrements in spatial contrast, respectively, by encoding the photon catch in the center cone relative to the photon catch in surrounding cones.

However, their role in encoding black and white percepts has been difficult to reconcile with their classification as "chromatic" neurons (reviewed in Patterson et al., 2019²⁸⁴). From an individual midget ganglion cell's spike output, it is impossible for a downstream neuron to distinguish whether the ganglion cell responded to an edge defined by a change in intensity or spectral contrast. Our intuition is that the midget ganglion cell's spectral and spatial opponency must be separated downstream, so that responses to changes in wavelength result in hue percepts while responses to changes in intensity result in black and white percepts. Models proposing midget ganglion cells mediate black-white typically assume this downstream separation (or "demultiplexing") must occur⁸⁹. However, the results of saturation discrimination experiments are contrary to our intuition - if changes in wavelength only resulted in hue percepts and changes in intensity only resulted in black-white percepts, saturation would not vary as a function of wavelength and the results of the

saturation scaling experiment in **Figure 5.1D** would be a constant 100% saturation (pure hue with 0% white percepts).

Here we demonstrated the saturation discrimination function can be accounted for by linear combinations of the outputs of midget ganglion cells, without assuming downstream “demultiplexing” occurs. In light of this result, we might consider an alternative: that the five types of ON and OFF midget ganglion cells illustrated in **Figure 5.3** are “labeled lines” for white and black, or more accurately, edges defined by increments and decrements in spatial contrast, respectively, and that other ganglion cells mediate hue perception^{311,44,268}.

An inability to distinguish between edges defined by intensity and spectral contrast is not a problem if the midget ganglion cell’s only function is only to encode edges (and not hue). After all, an optimal edge detector should be able to detect all edges, including the equiluminant ones commonly found in natural scenes¹⁵¹. A consequence is that the difference in spectral tuning between center and surround receptive fields will endow the edge detection that defines our percepts of black and white with a clear spectral sensitivity. For example, an ON midget ganglion cell will respond more to an edge between a broadband light and a 570 nm light than an edge between a broadband light and a 450 nm light (**Figure 5.4D, 5.5B, 5.5D**), thus signaling greater spatial contrast and a stronger white sensation for the 570 nm edge. Put simply, the striking wavelength-dependence of saturation can be explained at the level of the retinal output by spectrally-opponent edge detectors.

CHAPTER 6

A Color Vision Circuit for Non-Image-Forming Vision in the Primate Retina

The study presented in this chapter has been published as:

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6.1 Summary

Melanopsin-expressing, intrinsically photosensitive retinal ganglion cells (ipRGCs) synchronize our biological clocks with the external light/dark cycle²³. In addition to photoentrainment, they mediate the effects of light experience as a central modulator of mood, learning and health²²². This makes a complete account of the circuitry responsible for ipRGCs' light responses essential to understanding their diverse roles in our wellbeing. Considerable progress has been made in understanding ipRGCs' melanopsin-mediated responses in rodents^{252,22,387}. However, in primates, ipRGCs also have a rare blue-OFF response mediated by an unknown short wavelength sensitive (S) cone circuit⁷³. Identifying this S-cone circuit is particularly important as ipRGCs mediate many of the wide-ranging effects of short-wavelength light on human biology. These effects are often attributed to melanopsin, but there is evidence for an S-cone contribution as well^{304,138}. Here, we tested the hypothesis that the S-OFF response is mediated by the S-ON pathway through inhibitory input from an undiscovered S-cone amacrine cell. Using serial electron microscopy in the macaque retina, we reconstructed the neurons and synapses of the blue cone connectome, revealing a novel inhibitory interneuron, an amacrine cell, receiving excitatory glutamatergic input exclusively from S-ON bipolar cells. This

S-cone amacrine cell makes highly selective inhibitory synapses onto ipRGCs, resulting in a blue-OFF response. Identification of the S-cone amacrine cell provides the missing component of an evolutionarily-ancient circuit using spectral information for non-image forming visual functions.

6.2 Results

6.2.1 3D Reconstruction of the Primate S-Cone Connectome

Primate ipRGCs have a rare yellow-ON, blue-OFF color tuning and respond to increased activity in L- and M-cone pathways and decreased activity in S-cone pathways (i.e. LM-ON/S-OFF cone opponency)⁷³. The neural basis for the ipRGC’s color tuning remains an open question. The S-OFF responses are blocked by L-AP4, an ON pathway agonist, indicating an amacrine cell inhibition may invert the output of S-ON bipolar cells^{267,54,253,77}. However, the mammalian retina contains 20-40 amacrine cell types, making selective identification of one undiscovered type difficult^{287,235}. Moreover, due to the rarity of S-cones³¹², little is known about the diversity of pathways carrying S-cone signals. To investigate the circuit mechanisms responsible for the ipRGC’s S-OFF response, we used serial electron microscopy (EM) to reconstruct the neurons and synapses of the primate S-cone connectome from a volume of macaque inferior retina at ~ 1 -1.5 mm eccentricity, near the peak density for ipRGCs²²⁹. The resulting dataset of nearly 2000 sections spanned from the outer nuclear layer to the nerve fiber layer, enabling us to reconstruct complete circuits in 3D and identify the synaptic contacts between neurons.

Reliable identification of upstream S-cone circuitry is essential for identifying a novel S-cone neuron, thus, we began by reconstructing the established S-cone connectome, building on our previous reconstructions of S-cone bipolar cells in the outer retina²⁸². Briefly, S-cones were distinguished from L/M-cones by their highly stereotyped post-synaptic contacts¹⁹³. Each S-cone provides input to one to three S-ON bipolar cells and a single OFF midget bipolar cell (**Figure 6.1A**). The S-ON bipolar cells are highly cone-type specific and contact multiple S-cones, often passing multiple L/M-cones without contact to reach neighboring S-cones²⁴⁰. Using these features, we confidently identified eight S-cones and 14 S-ON bipolar cells.

Here, we extended these S-ON pathway reconstructions to the inner retina. The terminals of each bipolar cell type stratify at a stereotyped location within the inner retina, providing excitatory glutamatergic input to co-stratifying amacrine and retinal ganglion cells (RGCs) within the same

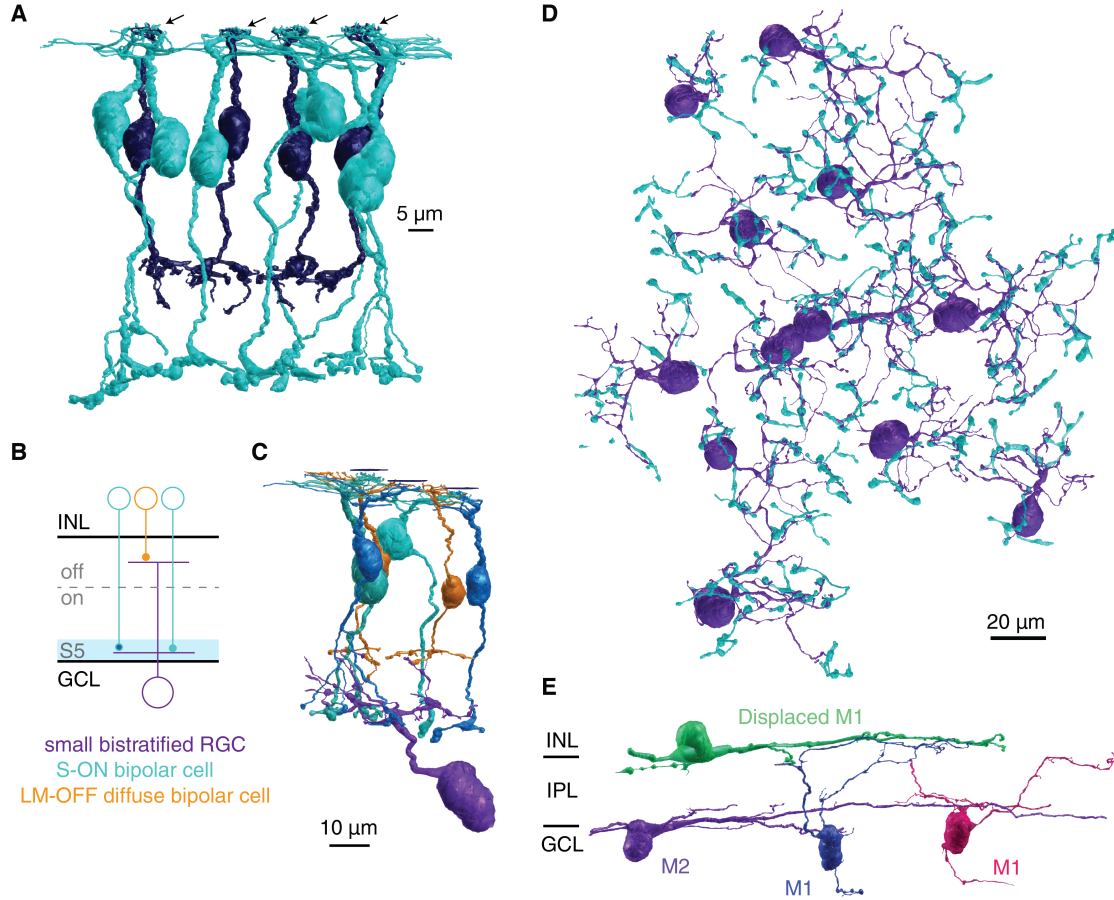


Figure 6.1 Serial EM reconstruction of ipRGCs and the S-cone connectome in primate retina. (A) Four representative S-cones (arrows) and their S-ON bipolar cell (blue) OFF midget bipolar cell (purple) contacts. (B) Small bistratified RGC circuit used for verification of S-cone and S-ON bipolar cell identification. (C) 3D reconstruction of the small bistratified RGC circuit in B. (D) 60 S-ON bipolar cell terminals contacting 12 small bistratified RGCs. (E) 3D reconstructions of the major ipRGC subtypes in primate retina. Note the ipRGC dendrites are monostratified but appear curved as the volume slopes away from the fovea.

layer¹¹⁶. Each of the 14 S-ON bipolar cells stratified in the innermost layer of the inner retina, sublamina 5 (S5), closest to the ganglion cell layer (GCL)²⁴⁰ (**Figure 6.1A, 1B**). We further verified S-ON bipolar cell identity by reconstructing their best known output: small bistratified RGCs (**Figure 6.1B, 1C**)^{71,46}. Each S-ON bipolar cell contacted two or three small bistratified RGCs and the most complete small bistratified RGCs collected inputs from up to nine S-ON bipolar cell terminals. The S-cone exclusive inner dendritic tier of each small bistratified RGC served as a bridge within the inner retina to identify an additional 46 S-ON bipolar cell terminals. In total, we reconstructed 60 S-ON bipolar cell terminals, each confirmed by extensive synaptic contacts with least one of 12 small bistratified RGCs (**Figure 6.1D**).

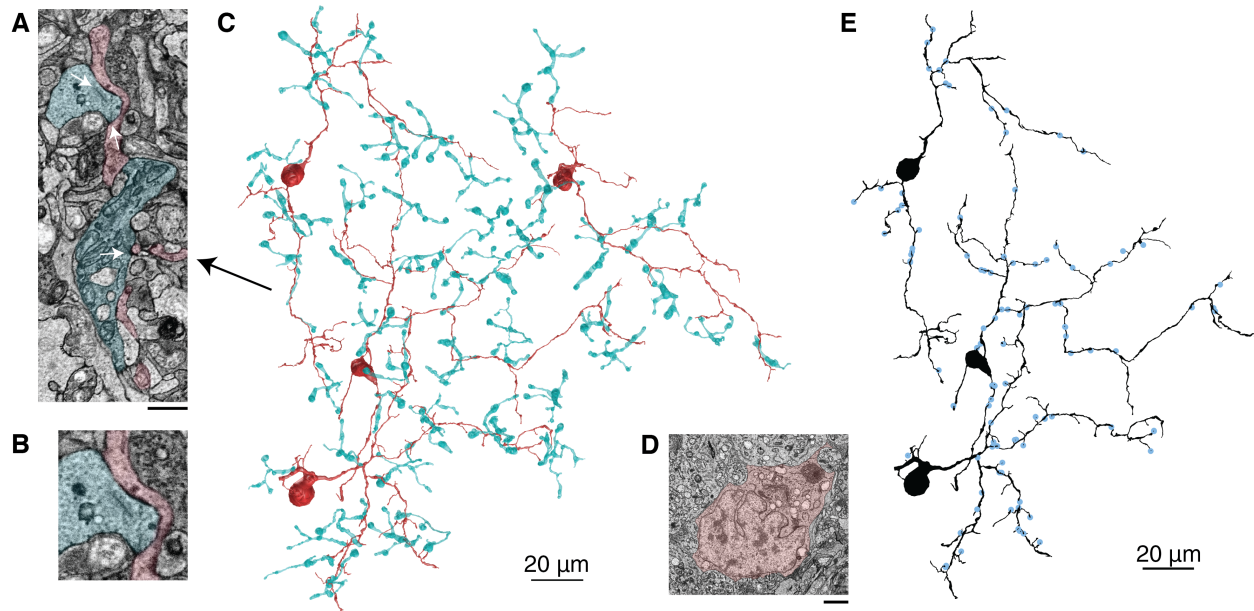


Figure 6.2 Identification of an S-cone exclusive amacrine cell. (A) Electron micrographs of three ribbon synapses (white arrows) from an S-ON bipolar cell (cyan) to an S-cone amacrine cell (red). Scale bar is 1.5 μm . (B) Inset from A showing two S-ON bipolar cell ribbon synapses. (C) 3D reconstructions of S-ON bipolar cell axon terminals providing input to four S-cone amacrine cells (red). (D) S-cone amacrine cells are easily identifiable by their asymmetric nuclei offset from the center of their soma. Scale bar is 1 μm . (E) Locations of S-ON bipolar cell input (blue) to three S-cone amacrine cells.

6.2.2 A novel S-cone selective amacrine cell links S-ON bipolar cells to ipRGCs

We next reconstructed the two major ipRGC subtypes reported in the primate retina, which are distinguished by their stratification in the outermost and innermost edges of the inner retina and are thought to correspond to M1 and M2 ipRGCs, respectively^{229,263,150} (**Figure 6.1E**). The dendrites of both ipRGCs branched sparsely, with dendritic fields extending beyond the edges of the volume. We focused our efforts on M1 ipRGCs with somas in the GCL and dendrites stratifying primarily in the outermost layer of the inner retina, sublamina 1 (S1). As shown in **Figure 6.3J**, this morphology limits the opportunities for contact with amacrine cells receiving S-ON bipolar cell input in S5.

Taken together, our reconstructions of the established S-cone circuitry and ipRGCs provided the necessary infrastructure to effectively search for the source of the S-OFF response. To identify candidate S-cone amacrine cells, we reconstructed neurons post-synaptic to the ribbon synapses in S-ON bipolar cell terminals. While many amacrine cells stratifying in S5 received occasional S-ON bipolar cell input, a population of medium-field displaced amacrine cells recognizable by their

distinctive soma ultrastructure (**Figure 6.2D**) were ultimately identified as exclusively contacting S-ON bipolar cells (**Figure 6.2C**). In the inner retina, the amacrine cells' thin, sparse processes co-stratified narrowly with the S-ON bipolar cell terminals in S5. Their dendritic fields covered the S-ON bipolar cell terminal mosaic, each collecting input from over ten S-ON bipolar cell terminals. Of the 102 bipolar cell inputs to the three most complete amacrine cells, all but six were from confirmed S-ON bipolar cells.

Amacrine cell processes are typically confined to the inner retina, however, some S-cone amacrine cells also extended processes into the GCL. Strikingly, these processes contacted an M1 ipRGC soma (**Figure 6.3A, Video S1**). Moreover, thin branches extended from the ipRGC's cell body to receive additional input from the S-cone amacrine cell processes. Small branches extending from the primary dendrites were also observed receiving input from S-cone amacrine cells within the inner retina (**Figure 6.3F, 6.3G**).

6.2.3 M1 ipRGCs receive targeted synaptic input from S-cone amacrine cells

We next examined the synaptic connections between S-cone amacrine cells and ipRGCs, focusing on the three most complete reconstructions. Conventional synapses, which are traditionally assumed to be inhibitory, were identified as a cluster of vesicles on the pre-synaptic neuron adjacent to a membrane density on the post-synaptic neuron. **Figure 6.3B - 6.3E** and **6.3H** show S-cone amacrine cell synapses onto the ipRGC soma and branches within the inner retina and GCL. This unique concentration of S-cone-exclusive inhibition near the action potential initiation site could provide modulatory control over synaptic inputs received throughout the ipRGC's dendritic field²³². The somas of human ipRGCs and other RGCs have been reported to receive GABAergic input^{150,196,197} and the S-cone amacrine cell's morphology is consistent with a GABAergic amacrine cell³⁷⁷. Moreover, this motif is distinct from the previously identified glycinergic S-cone amacrine cell in ground squirrel that stratifies diffusely, collecting S-ON bipolar cell input in S5 and providing S-cone inhibition to non-ipRGC ganglion cells stratifying in S1^{54,330}. Based on strong morphological similarities, stratification and displaced soma, the primate S-cone amacrine cell is likely the homolog to the MA-S5 amacrine cell in mouse⁸⁴ and the A12 amacrine cell in human, cat and ground squirrel¹⁹⁴.

To visualize the extent of S-cone convergence onto M1 ipRGCs, we plotted the network of upstream S-cone exclusive circuits (**Figure 6.3I**). Each S-ON bipolar cell collects input from an

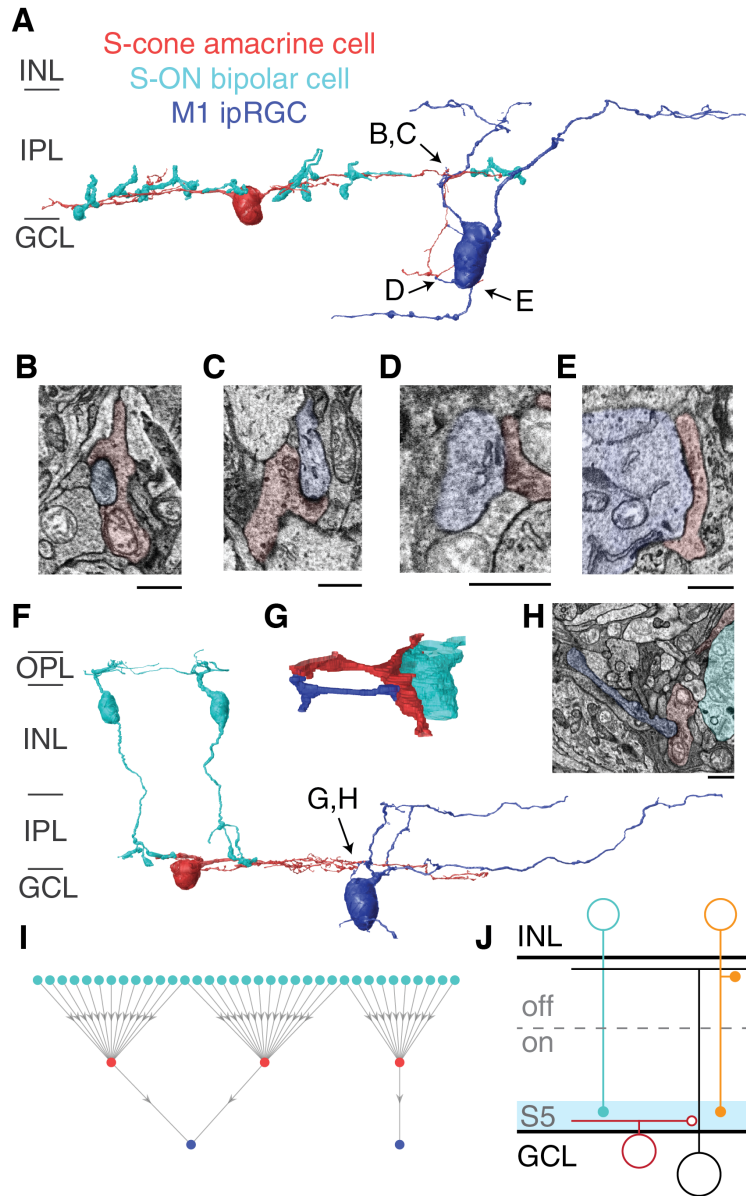


Figure 6.3 M1 ipRGCs receive targeted inhibitory input from S-cone amacrine cells. (A) 3D reconstruction of an M1 ipRGC receiving synaptic input from an S-cone amacrine cell. The S-cone amacrine cell dendrite travels into the ganglion cell layer to provide additional synaptic input to the M1 ipRGC soma. See also **Video S1**. (B-E) Four electron micrographs of the synapses in A. (F) 3D reconstruction of S-cone amacrine cell input to an M1 ipRGC, with complete reconstructions of two representative S-ON bipolar cells. (G) Detailed reconstruction of S-cone amacrine cell to ipRGC synaptic contact. (H) Synapse from an S-cone amacrine cell to the M1 ipRGC in F and H. Scale bar is 1 μm . (I) Network of S-ON bipolar cells, S-cone amacrine cells and M1 ipRGCs. (J) Circuit diagram of cone-opponent inputs to ipRGCs. *En passant* synapses from ON diffuse bipolar cells (orange) contribute to the LM-ON response^{145,229}.

overlapping set of 2-3 S-cones, as in **Figure 6.1A**. The network in **Figure 6.3I** is likely an underestimate at both the level of S-ON bipolar input to S-cone amacrine cells and S-cone amacrine cell input to ipRGCs, as we only included ipRGCs with somas within our EM volume and occasional S-cone amacrine cell processes extended past the edges of the volume or could not be reliably annotated. Nevertheless, the high degree of S-cone convergence suggests S-cone amacrine cell input to M1 ipRGCs contributes a strong S-cone signal.

6.3 Conclusions

Short-wavelength cone exclusive ON bipolar cells are a highly conserved feature of mammalian retinas^{295,156} forming the basis of the primordial color vision circuit comparing long and short-wavelength light²⁵⁴. Here, we show that this circuit extends to the inner retina, with an additional S-cone exclusive neuron, an amacrine cell receiving only S-ON bipolar cell input. The concept of a primordial color vision circuit has been considered as the precursor to the primate hue perception circuitry, however, our findings suggest this ancient color vision circuit is instead still serving its original function. Much of the S-cone amacrine cell's output is targeted to ipRGCs, another highly conserved neuron mediating ancient non-image-forming visual functions. Accordingly, the S-cone amacrine cell may be part of an evolutionarily ancient color vision circuit, not for hue perception, but for non-image-forming vision.

The downstream functions of the M1 ipRGCs are among the best characterized of all RGCs²³⁴. Thus, our results establish the S-cone amacrine cell among the few amacrine cell types to be placed within the context of a specific retinal circuit with an established visual function. The high degree of cone specificity demonstrated in **Figure 6.2C** indicates a crucial role for the S-cone amacrine cell. For example, the evolutionary pressure to know the time of day is strong and color-opponent circadian photoentrainment is well-established in many vertebrates^{341,258}. As the sun rises and sets, the spectral contrast of the sky changes dramatically, with peaks in L/M- vs. S-cone contrast at sunrise and sunset^{386,338,285}. The S-cones providing input to the S-cone amacrine cell receive feedback from L/M-cones, effectively encoding the S-cone contrast relative to L/M-cone contrast²⁷⁸. This cone-opponent signal is conveyed to inner retinal neurons by the S-ON bipolar cells¹²³. Thus, the output of the S-cone amacrine cell is predicted to be color-opponent (S-ON/LM-OFF) and is well-suited to encode the reliable circadian changes in the color of the sky.

6.4 Methods

Key Resources Table.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Macaque retina	Regional Primate Research Center	N/A
Software and Algorithms		
Illustrator	Adobe	https://adobe.com
MATLAB	Mathworks	RRID: SCR_001622
Nornir	Marc/Jones Lab, Utah	RRID: SCR_003584
SBFSEM-Tools	Neitz Lab, Washington	RRID: SCR_017350
Viking	Marc/Jones Lab, Utah	RRID: SCR_005986

Lead Contact and Materials Availability. Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Jay Neitz (jneitz@uw.edu). This study did not generate any new unique reagents.

Experimental Model and Subject Details. Retinal tissue was obtained from a terminally anesthetized male macaque (*Macaca nemestrina*) monkey through the Tissue Distribution Program at the Washington National Primate Research Center. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Washington.

Microscopy. The tissue was imaged using a Zeiss Sigma VP field emission scanning electron microscope equipped with a 3View system and sectioned in the horizontal plane. Tissue preparation and image collection were optimized in signal-to-noise ratio for visualizing small, low contrast features such as synaptic ribbons that have previously been a challenge for serial block-face scanning (**Figure 6.2A, 6.2B**). In each 90 nm section, an area approximately 200 μm on a side was imaged as a 5 X 5 montage at a resolution of 7.5 nm/pixel. The volume contained 1893 horizontal 90 nm sections from the ganglion cell layer through the cone pedicles. Image registration was performed

using Nornir (<http://nornir.github.io>).

Tissue Preparation. A block of inferior parafoveal retinal tissue at ~ 1 mm eccentricity from the fovea center was processed as previously described⁹². A transmission electron microscopic image of a cross-section of the retina can be seen in Figure 1A of our previously published work²⁸². Briefly, the eyecup was placed in 4% glutaraldehyde in 0.1M sodium cacodylate buffer pH 7.4 and while in this solution a 1 mm square of retina centered 1.5 mm inferior to the center of the fovea was cut out and then fixed overnight at 4°C. The tissue was next washed 5 x 5 min in 0.1M cacodylate buffer, then post fixed in osmium ferrocyanide for 1 h on ice. The tissue was next washed 5 x 5min in double distilled (dd)H₂O at room temperature (RT) and incubated in a 1% thiocarbohydrazide solution for 20 min at RT. The tissue was washed 5 x 5min in ddH₂O and placed in 2% osmium tetroxide for 30 min at RT. The tissue was next washed 5 x 5min in ddH₂O and en block stained in 1% uranyl acetate, (aqueous), overnight in the refrigerator. The next day the tissue was washed 5 x 5 min in ddH₂O, then en bloc stained in Walton’s lead aspartate for 30 min at 60°C. The tissue as next washed 5 x 5 min in ddH₂O and dehydrated in ice cold 30%, 50%, 70%, and 95% ETOH, then allowed to come to RT. This was followed by 2 changes of 100% ETOH and two changes of propylene oxide. The tissue was then infiltrated in a 1:1 mixture of propylene oxide:Durcupan resin, for 2 h and then infiltrated overnight in fresh Durcupan. The next day the tissue was given a fresh change of Durcupan for two h and then placed in flat embedding molds and polymerized in a 60°C oven for two days. The block was then trimmed to approximately 0.5 mm². At this eccentricity (the edge of the foveal slope), the displacement of RGCs from cone pedicles was minimized while still remaining in a region where most midget RGCs receive single cone input.

Annotation. The serial EM volumes were annotated using the web-based, multiuser Viking software described previously (<http://connectomes.utah.edu>)⁵. Neuronal processes were traced through the sections by placing a circular disc at the structure’s center of mass and linking the disc to annotations on neighboring sections. Compared to contouring⁴⁸ and skeleton reconstruction³⁵, this connectomics approach provided an optimal balance of efficiency and resolution for circuit-level research questions^{5,29} [45,48]. There were two exceptions to the disc annotations. Cone pedicles were outlined using a closed curve polygon defined by three or more control points. The detailed

morphological reconstruction in **Figure 6.3G** was obtained by manually tracing the outlines of each dendritic process.

Synapses were annotated with lines connected by 2-3 control points and linked to a parent neuron. Synapse identification used previously described parameters¹⁰⁵. The boundaries of the inner plexiform layer were determined locally and marked throughout the volume with a total of 258 markers for the inner nuclear layer (INL) boundary and 453 markers for the IPL-ganglion cell layer (GCL) boundary.

Data Analysis and Visualization. Data analysis and 3D rendering were performed using SBFSEM-tools, an open-source Matlab (Mathworks) program developed in the Neitz lab (<https://github.com/neitzlab/sbfsem-tools>)^{282,29}. Final figures were compiled in Adobe Illustrator.

The cone pedicle locations in **Figure 6.1A** were based on XYZ coordinates of the closed curve annotation control points, connected by Catmull-Rom splines. The detailed morphology reconstructions in **Figure 6.3G** was obtained by extracting the coordinates from a stack of manually annotated processes. These coordinates were used to build a volume from which isosurfaces were extracted with a marching cubes algorithm then rendered as a triangle mesh. All other analyses and visualizations were performed using the X, Y, Z coordinates and radius of each annotation. The 3D models are triangle meshes built by rendering segments of connected annotations as rotated cylinders centered at each annotations' XYZ coordinates and scaled by their radii.

INL-IPL and GCL-IPL boundary surfaces were fit to the X, Y and Z coordinates of each IPL boundary marker type using bicubic interpolation. IPL depth could then be calculated for each annotation individually. Given an annotation's X, Y coordinates, the surfaces supplied the depth of the IPL boundaries at that X,Y location. The annotation's Z coordinate relative to the Z coordinates of each boundary could be calculated to determine percent IPL depth.

Cell Type Identification. In addition to the S-ON bipolar cell verification described in the text and our previous work²⁸², we also verified the S-ON bipolar cells were distinct from the other two bipolar cell inputs stratifying in S5. The morphology of our DB6 reconstructions matched previous reports^{145,359}. A reliable parameter for distinguishing S-ON and DB6 bipolar cells is the smaller diameter of their axon terminal branches. Rod bipolar cell terminals were distinguished by input

exclusively from rod photoreceptors in the outer retina and an absence of ribbon synapses onto RGCs in the inner retina.

To verify the identity of outer-stratifying (M1) ipRGCs, our strongest criteria was the dendritic stratification depth at the IPL-INL border^{263,150,183}. Of all reported primate RGCs, M1 ipRGCs stratify closest to the inner nuclear layer (giant sparse monostratified⁶⁹). The next closest RGC to the inner nuclear layer is the large sparse monostratified RGC, which stratifies around 30% IPL depth^{69,99}. The M1 ipRGCs in this study stratified between 0-20%. Some primary dendrites of the M1 ipRGCs with somas in the ganglion cell layer travelled through S5 for tens of microns before descending to S1. However, all primary dendrites either reached S1 or ran off the edge of the volume. Displaced M1 ipRGCs are the only known RGC with a soma in the INL, providing further confirmation for the displaced M1 ipRGC (green) in **Figure 6.1E**, which stratified closest to the INL, had a large soma with significant synaptic input from a single amacrine cell type and a single axon with an ultrastructure and morphology matching the axons of RGCs in the ganglion cell layer. We also confirmed the presence of previously reported synaptic inputs. M1 ipRGCs receive some ON bipolar cell input in S1 from *en passant* synapses from DB6 bipolar cell axons, as well as other non-DB6 bipolar cell input presumed to be from DB1 bipolar cells¹⁴⁵. We observed both M1 ipRGCs receiving infrequent bipolar cell input in S1 from neurons matching the morphology of DB1 bipolar cells^{358,299}. Several DB6 bipolar cell axons were reconstructed, however, *en passant* ribbon synapses in bipolar cell axons are smaller than ribbon synapses in bipolar cell terminals[?] and were difficult to reliably identify with our volume's resolution. No contacts were observed between ipRGC dendrites and S-ON bipolar cells or the S-cone OFF midget bipolar cells identified in our previous work²⁸². In one case, appositions between a rod bipolar cell and an M1 ipRGC dendrite were observed^{150,277}. As previously reported, a single type of amacrine cell made large inhibitory synapses along the dendrites of M1 ipRGCs and the soma of the displaced M1 ipRGC. While none were traced back to a soma, their morphology was consistent with dopaminergic amacrine cells reported to provide extensive synaptic input to M1 ipRGC somas and dendrites²²⁹.

Quantification and Statistical Analysis. No statistical tests were used in the manuscript.

Data and Code Availability. The 3D reconstructions from Viking Viewer annotations are visualized with SBFSEM-tools, an open-source MATLAB toolbox (<https://github.com/neitzlab/sbfsem-tools>). The Viking Viewer software for visualizing both the dataset and the annotations is freely available (<http://connectomes.utah.edu>). Raw data and additional analysis code will be provided upon request by the Lead Contact, Jay Neitz (jneitz@uw.edu).

6.5 Supplementary Information

A supplementary video can be found at <https://www.sciencedirect.com/science/article/pii/S0960982220300841>.

Video S1 S-cone Amacrine Cell Circuit Built from EM Images, Related to Figure 3A. The circuit in **Figure 6.3A** is shown along with the corresponding EM images used to reconstruct the neurons.

CHAPTER 7

Another Blue-ON Ganglion Cell in the Primate Retina

The study presented in this chapter is under review for publication as:

Patterson, S.S., Mazzaferri, M.A., Bordt, A.S., Chang, J., Neitz, M. and Neitz, J. Another Blue-ON Ganglion Cell in the Primate Retina.

7.1 Summary

The primate retina’s strategy for color coding is often described as redundancy reduction, with the three most common ganglion cell types capturing much of the variance in natural spectra. Within this compact code, “Blue-ON” small bistratified ganglion cells were thought to be the only pathway necessary for comparing long- and short-wavelengths. Here, we identify a wide-field ganglion cell receiving the same cone-opponent input, challenging the underlying hypotheses of classic color vision models.

7.2 Introduction

A classic and highly influential model of visual processing proposes that the role of the retina is to compress visual information for optimal transmission to the brain¹³. Drawing on ideas from information theory, an efficient retinal code could be defined as one that reduces redundancy to communicate as much information as possible, given the optic nerve’s limited capacity. From this redundancy reduction hypothesis, a theory of retinal color coding emerged in which the three most common retinal ganglion cell (RGC) types captured much of the variance in natural spectra^{38,9}.

Within this compact code, the “Blue-ON” small bistratified RGC was thought to be the only pathway necessary for comparing short (S) wavelength-sensitive cones to long (L) and medium (M) wavelength-sensitive cones^{71,89}. Here, we discovered a new wide-field RGC type receiving the same cone-opponent input as the small bistratified RGC, indicating that there is more redundancy in the retinal color code than previously appreciated.

7.3 Results

To explore S-cone circuit diversity, we reconstructed the neurons and synapses of the S-cone connectome from a serial electron microscopy volume of macaque central retina. Building on our previous work²⁸³, we reconstructed 70 S-ON bipolar cell (BC) terminals providing excitatory ribbon synaptic input to 20 small bistratified RGCs (**Figure 7.1A**). This extensive network of well-studied S-cone circuits provided a base for confident identification of novel S-cone neurons. The small bistratified RGC’s “Blue-ON” response is more specifically S-ON/LM-OFF cone opponency⁷¹. S-ON BC ribbon synaptic input provides both the S-ON and some of the LM-OFF response (**Figure 7.1F**)¹²³. Accordingly, other neurons receiving the same S-ON BC input are predicted to have similar spectral tuning.

We began by mapping the different RGC types post-synaptic to S-ON BC ribbon synapses. The majority were small bistratified RGCs; however, as previously reported⁴⁶, we also encountered other RGC types (**Figure 7.1B**). By reconstructing these unknown neurons, we identified a wide-field monostратified RGC type that receives nearly exclusive excitatory input from S-ON BCs. These wide-field RGCs had large dendrites that branched sparsely in sublamina 5, directly adjacent to the ganglion cell layer, and extended beyond the edges of the 220 x 220 micron volume (**Figure 7.1C, 7.1D**). Occasionally, smaller processes were observed extending to S-ON BC terminals, then stopping abruptly after receiving ribbon synaptic input. We exhaustively reconstructed the axon terminals of all presynaptic BCs to the two most complete wide-field RGCs and confirmed the identity of all but two presynaptic BCs. Each wide-field RGC received just 3.28 ± 2.64 ribbon synapses from each S-ON BC (mean $\pm$ s.d., n=43), yet the total S-cone input to the two wide-field RGCs was substantial as each received input from 23 and 26 S-ON BCs, respectively (**Figure 7.1E**). In total, S-ON BCs provided 94.63% of the identified BC input to the two wide-field RGCs (139 of 147 synapses), with the remaining input from ON midget and DB6 BCs.

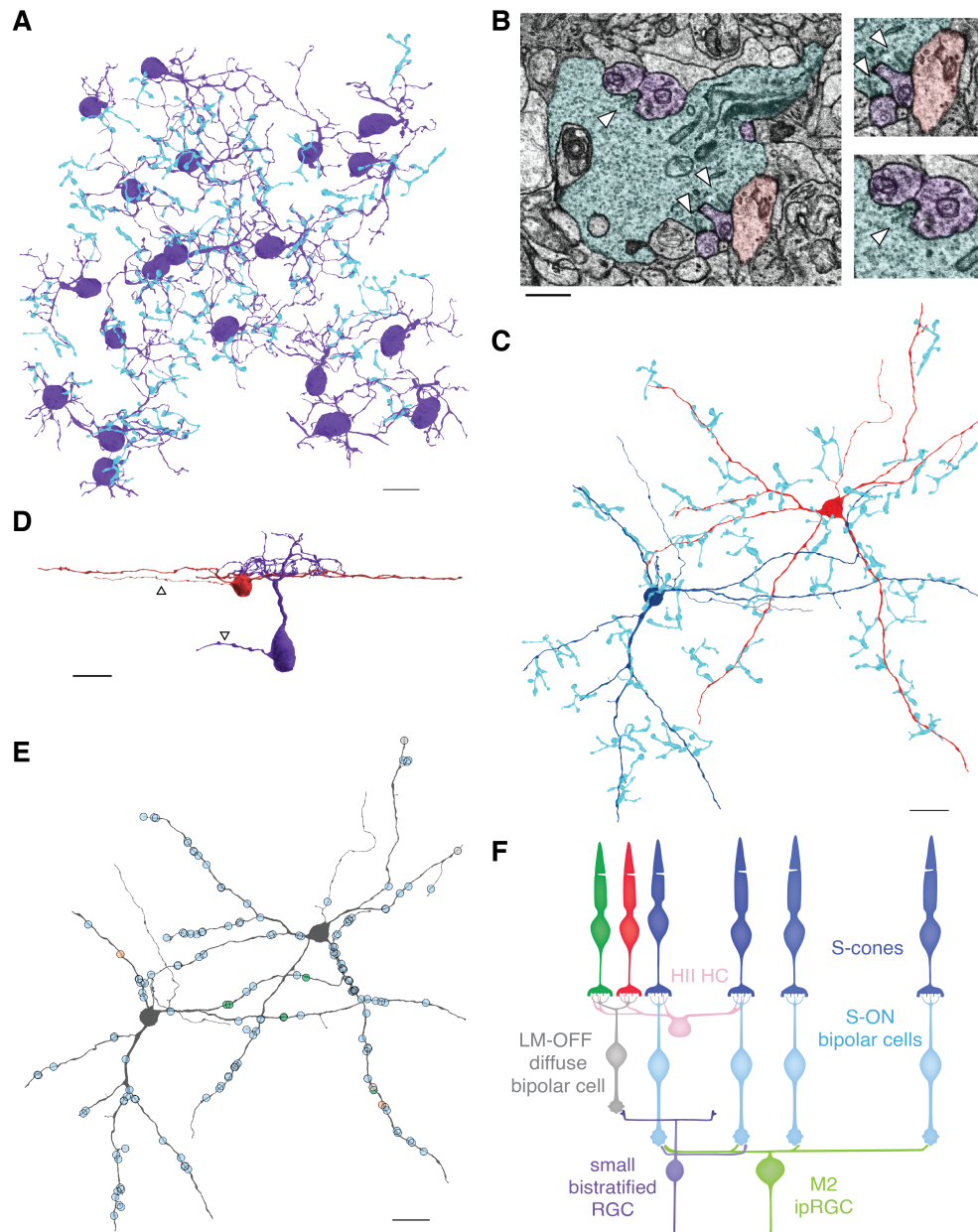


Figure 7.1 Connectomic reconstructions of two distinct types of “Blue-ON” RGC. (A) Reconstructions of S-ON BC axon terminals (blue) providing input to small bistratified RGCs (purple). (B) Electron micrograph of an S-ON BC terminal (blue) and post-synaptic dendrites of small bistratified RGCs (purple) and a wide-field RGC (red). Arrowheads mark presynaptic ribbons within the S-ON BC terminal, which are adjacent to a membrane density on the postsynaptic neuron. (C) Reconstructions of two wide-field RGCs (dark blue, red) and presynaptic S-ON BC terminals (blue). (D) 3D reconstructions of a small bistratified RGC (purple) and wide-field RGC (red) viewed from the side. (E) Locations of ribbon synaptic input to the wide-field RGCs. S-ON BC, ON midget BC and DB6 BC inputs are marked in blue, green and orange, respectively. (F) Circuit diagrams for the wide-field RGCs and small bistratified RGCs. The S-ON bipolar cell carries an LM-OFF response contributed by HII horizontal cell-mediated antagonistic feedback to S-cones from neighboring L/M-cones²⁷⁸. The scale bar in B is 2 microns and the rest are 20 microns.

Interestingly, there is a previous description of S-ON BC input to a wide-field monostратified RGC in primate; the first report of S-ON BCs also mentioned contacts with an RGC resembling those in **Figure 7.1C**²⁴⁰. Based on the morphology and stratification of known primate RGC types, the wide-field RGCs in **Figure 7.1C** correspond to the M2 intrinsically photosensitive retinal ganglion cells (ipRGCs)^{283,146}. Compared to M1 ipRGCs, M2s have more BC input, lower melanopsin expression and are primarily driven by their synaptic inputs^{327,145}. Still, the S-ON BC input identified here is surprising as it confers the opposite spectral tuning as that reported for M1 ipRGCs⁷³. However, this result is consistent with the different spectral tunings reported functionally for human ipRGC subtypes²⁶¹. S-ON BC input to M1 ipRGCs was not observed and the S-cone amacrine cells mediating the M1 ipRGC’s S-OFF responses rarely contacted M2 ipRGCs (**Figure 7.2**)²⁸³.

Primate ipRGCs project to the suprachiasmatic nucleus, olivary pretectal nucleus (OPN), and, like the small bistratified RGCs, the lateral geniculate nucleus^{73,149}. Their projections to the OPN drive the pupillary light reflex, which responds at both the onset and offset of S-cone isolating lights⁴⁰¹. M1 ipRGCs are proposed to contribute to the OFF response, and S-ON BC input to M2 ipRGCs could explain the ON response. More generally, this circuit provides an additional candidate neural substrate for the wide-ranging effects of short-wavelength light on ipRGC-mediated non-image-forming visual functions essential to human health, which were originally attributed solely to melanopsin.

7.4 Discussion

Small bistratified RGCs were long thought to be the only pathway carrying S-cone signals from the primate retina, but here we identified another RGC type with the same S-ON BC input. Accordingly, the new wide-field RGC is predicted to share the small bistratified RGC’s S-ON/LM-OFF spectral tuning (**Figure 7.1F**). In theory, the wide-field RGCs’ large spatial receptive field and spectral tuning could have been obtained downstream by pooling the outputs of the small bistratified RGCs. Why then have multiple pathways carrying “redundant” spectral signals? Importantly, the spectral tuning of the two “Blue-ON” RGCs is only redundant if considered independently from each RGCs’ other response properties, such as their spatial scale, intrinsic photosensitivity and OFF BC input (**Figure 7.1D, 7.1F**). In reality, these properties must be considered together as they operate

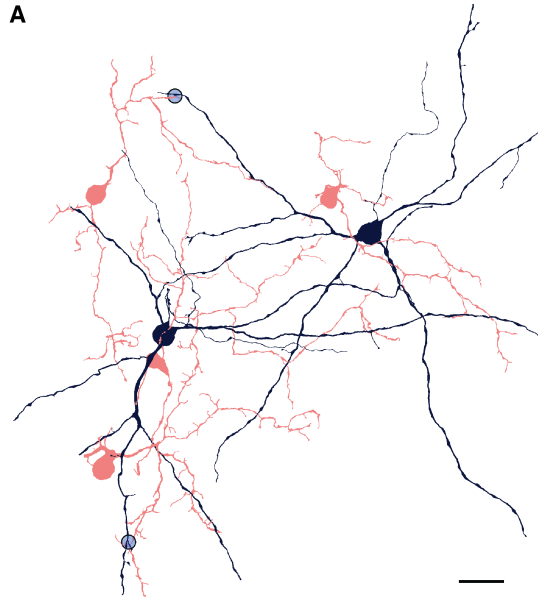


Figure 7.2 S-cone amacrine cells do not receive substantial S-cone amacrine cell input. (A) The two wide-field RGCs (navy) and four previously published S-cone amacrine cells (red), which provide M1 ipRGCs with an S-OFF response. Locations of S-cone amacrine cell input to the wide-field RGCs are marked in blue²⁸³. Scale bar is 20 microns.

simultaneously and in concert to shape each RGC type’s final spike output. A simple interpretation of the differing upstream circuitry and downstream projections two “Blue-ON” RGCs is that they reflect different roles in vision. However, this interpretation does not fit into canonical models of primate vision built on the hypothesis that the sole goal of retinal coding is to reduce redundancy⁹.

The redundancy reduction hypothesis predicts that an efficient retinal code maximizes information transmission through a small number of RGCs with decorrelated responses. This inability to account for the diversity of RGC types and the lack of independence in their responses has driven the development of new hypotheses^{120,15}. However, these ideas remain the foundation of the leading primate color vision models⁸⁹, which may need to be critically revisited. While spectral opponency undoubtedly reduces redundancy, this alone produces an impoverished and inaccurate view of retinal color coding¹³⁵. Accounting for both “Blue-ON” RGC types may also require the context of the visual functions each RGC evolved to mediate¹².

7.5 Methods

Key Resources Table.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Macaque retina	Regional Primate Research Center	N/A
Software and Algorithms		
Illustrator	Adobe	https://adobe.com
Photoshop	Adobe	https://adobe.com
MATLAB	Mathworks	RRID: SCR_001622
Nornir	Marc/Jones Lab, Utah	RRID: SCR_003584
SBFSEM-Tools	Neitz Lab, Washington	RRID: SCR_017350
Viking	Marc/Jones Lab, Utah	RRID: SCR_005986

Lead Contact and Materials Availability. Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Jay Neitz (jneitz@uw.edu). This study did not generate any new unique reagents.

Experimental Model and Subject Details. Retinal tissue was obtained from a terminally anesthetized male macaque (*Macaca nemestrina*) monkey through the Tissue Distribution Program at the Washington National Primate Research Center. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Washington.

Tissue Preparation. A block of inferior parafoveal retinal tissue at ~ 1 mm eccentricity from the fovea center was processed as previously described⁹². Briefly, the eyecup was placed in 4% glutaraldehyde in 0.1M sodium cacodylate buffer pH 7.4 and while in this solution a 1 mm square of retina centered 1.5 mm inferior to the center of the fovea was cut out and then fixed overnight at 4°C. The tissue was next washed 5 x 5 min in 0.1M cacodylate buffer, then post fixed in osmium ferrocyanide for 1 h on ice. The tissue was next washed 5 x 5 min in double distilled (dd)H₂O at room temperature (RT) and incubated in a 1% thiocarbohydrazide solution for 20 min at RT. The tissue was washed 5 x 5 min in ddH₂O and placed in 2% osmium tetroxide for 30 min at RT. The tissue was again washed 5 x 5 min in ddH₂O and en bloc stained in 1% uranyl acetate, (aqueous),

overnight in the refrigerator. The next day the tissue was washed 5 x 5 min in ddH₂O, then *en bloc* stained in Walton’s lead aspartate for 30 min at 60°C. The tissue as next washed 5 x 5 min in ddH₂O and dehydrated in ice cold 30%, 50%, 70%, and 95% ETOH, then allowed to come to RT. This was followed by 2 changes of 100% ETOH and two changes of propylene oxide. The tissue was then infiltrated in a 1:1 mixture of propylene oxide:Durcupan resin, for 2 h and then infiltrated overnight in fresh Durcupan. The next day the tissue was given a fresh change of Durcupan for two h and then placed in flat embedding molds and polymerized in a 60°C oven for two days. The block was then trimmed to approximately 0.5 mm².

Microscopy. A cross-section of the retina taken with a transmission electron microscope prior to sectioning can be found in our previously published work²⁸². A serial block-face scanning electron microscope (Zeiss/Gatan) was used to section the tissue horizontally and image each section at a resolution of 7.5 nm/pixel. The final volume contained 1893 sections at 90 nm section widths and spanned from the ganglion cell layer through the cone pedicles. Image registration was performed using Nornir (RRID: SCR_003584, <http://nornir.github.io>) and supplemented with custom MATLAB (Mathworks) code.

Annotation. The serial EM volumes were annotated using the web-based, multi-user Viking software described previously (RRID: SCR_005986, <http://connectomes.utah.edu>)⁶. Neuronal processes were traced through the sections by placing a circular disc at the structure’s center of mass and linking the disc to annotations on neighboring sections. Synapses were annotated with lines connected by 2-3 control points and linked to a parent neuron. Conventional and ribbon synapse identification used previously described parameters¹⁰⁵.

Data Analysis and Visualization. Data analysis and 3D rendering were performed using SBFSEM-tools, an open-source MATLAB toolbox developed in the Neitz lab (RRID: SCR_017350, <https://github.com/neitzlab/sbfsem-tools>). The annotations associated with a neuron and the links between them were represented as an undirected graph. Triangle meshes were built by rendering segments of connected annotations as rotated cylinders centered at each annotation’s XYZ coordinates and scaled by their radii. To mark locations of synapses in **Figure 7.1E**, the coordinates of each synapse annotation were averaged to a single XYZ location. Adobe Photoshop

was used to enhance the contrast of the electron micrograph in **Figure 7.1B** and to add in color overlays marking relevant structures. Final figure layouts were produced in Adobe Illustrator.

Cell Type Identification. Bipolar cell inputs to the wide-field RGCs were identified by the presence of two features across multiple sections: a presynaptic ribbon structure and an adjacent post-synaptic membrane density¹⁰⁵. Of the 51 bipolar cells presynaptic to the wide-field RGCs, 49 could be classified as either an S-ON bipolar cell, ON midget bipolar cell or DB6 ON diffuse bipolar cell. The remaining two bipolar cells were located at the edges of the volume and could not be reconstructed sufficiently to confirm cell type. The different ON bipolar cell types could be readily distinguished by their morphology and the stratification of their axon terminals within the inner retina³⁵⁹. Further confirmation was obtained by reconstructing the RGCs post-synaptic to each bipolar cell. Each S-ON bipolar cell was confirmed either through contacts with S-cones and/or input to small bistratified RGCs. Each ON midget bipolar cell was confirmed by reconstruction of the post-synaptic ON midget RGC, which had characteristically small, dense dendritic fields that collected the majority of their bipolar cell input from just one or two ON midget bipolar cells¹⁹⁵. DB6 bipolar cells could be clearly distinguished from S-ON bipolar cells by their larger dendritic fields, thinner axon terminals and lack of input to small bistratified RGC dendrites.

Small bistratified RGCs were identified by their characteristic morphology (dense branching in sublamina 5 and sparse branches extending to sublamina 2), extensive S-ON bipolar cell input and mosaic tiling of their dendritic fields (**Figure 7.1A**). Some small bistratified RGCs are only partially complete as our goal in reconstructing them was to confirm the identity of nearby S-ON bipolar cell terminals. Both the small bistratified RGCs and the wide-field RGCs lacked both conventional and ribbon synaptic outputs, a distinguishing feature of RGCs.

Our classification of the wide-field RGCs as M2 ipRGCs expanded on our previously published criteria for ipRGC identification and reconstructions of M1 ipRGCs²⁸³. As described in the text, the wide-field RGCs' morphology and stratification was consistent with that of M2 ipRGCs (also called inner-stratifying ipRGCs or giant sparse RGCs)^{150,229,263} and did not match any other known primate RGC type^{69,146}. Notably, one of the wide-field RGCs had two axons extending from a major dendrite rather than the standard single axon from the soma, a rare feature described only for M1 and M2 ipRGCs^{229,180}. Consistent with previous reports, the M2 ipRGCs in our volume received

more bipolar cell input than the M1 ipRGCs^{272,145} and synaptic inputs were often found in clusters along the dendrites¹⁸³. In the past, this bipolar cell input to M2 ipRGCs has been attributed to DB6 bipolar cells. DB6 bipolar cell axons make en passant synapses onto M1 ipRGCs in the OFF layer and markers for synaptic output within DB6 axons terminals in the ON layer have been observed next to M2 ipRGCs dendrites¹⁴⁵. We only observed two instances of synaptic input from DB6 bipolar cells (orange circles in **Figure 7.1E**), although their ribbon synapses were often found in close proximity to the dendrites of the wide-field RGCs, possibly explaining the earlier results from immunohistochemistry.

The closest alternative correlate to the wide-field RGC is the ON large sparse RGC. However, several lines of evidence make a strong case against the wide-field RGCs in **Figure 7.1C** and **7.1D** being ON large sparse RGCs. First, the ON large sparse RGCs stratify closer to sublamina 4, between 60-80% depth within the inner retina (where 0% is the inner nuclear layer and 100% is the ganglion cell layer)²⁸⁸. The RGCs in our study stratified within sublamina 5 at >90% depth. Second, the dendrites of both wide-field RGCs extended beyond the edges of the volume; thus, their dendritic field diameter far exceeds that reported for ON large sparse RGCs at a similar eccentricity and is more consistent with M2 ipRGCs²⁸⁸.

Quantification and Statistical Analysis. The synaptic contacts between S-ON bipolar cells and M2 ipRGCs were analyzed with custom MATLAB code written for SBFSEM-tools (<https://github.com/neitzlab/sbfsem-tools>). The mean, standard deviation and N are reported in the text.

Data and Code Availability. The Viking View software for visualizing both the dataset and the annotations is freely available (<https://connectomes.utah.edu>). The 3D reconstructions from Viking Viewer annotations are visualized with SBFSEM-tools, an open-source MATLAB toolbox (<https://github.com/neitzlab/sbfsem-tools>). Raw data and additional analysis code will be provided upon request by the Lead Contact, Jay Neitz (jneitz@uw.edu).

CHAPTER 8

Conclusions

Color vision research has largely focused on the two most common cone-opponent neurons: midget RGCs comparing L- and M-cones (L vs. M) and small bistratified RGCs comparing S-cones to L- and M-cones (S vs. L+M). However, the models based on these neurons struggle to account for many aspects of color perception. Early surveys of color opponency in the retina and LGN reported a larger diversity of cone-opponent neurons, including other S vs. L+M neurons and even L vs. S+M and M vs. L+S neurons. These neurons were rare and, as a result, they eluded systematic investigation and were overlooked by most color vision models. Eventually, the small bistratified RGC and its projections to the “Blue-ON” LGN neurons were thought to be the only pathway for S-cone information to leave the retina, or at least the only pathway that could fit into a comprehensive model of primate vision.

This thesis first identified conditions where activity in S vs. L+M neurons, like the small bistratified RGC, does not elicit hue percepts, adding to the mounting evidence that they mediate visual functions other than hue perception. The following chapters revealed an unexpected diversity of S vs. L+M retinal circuits by confirming a controversial S-cone OFF midget circuit and by identifying S-cone amacrine cell input to M1 ipRGCs and S-ON bipolar cell input to M2 ipRGCs (**Figure 8.1**). To account for the surprising number of S vs. L+M RGCs, this thesis also explored new ways that wavelength information could be used by the primate visual system.

More generally, this work explored different perspectives on retinal function. In a recent essay on the unsolved mystery of vision, Masland & Martin (2007) presented “a portrait of a field that is ‘stuck’,” and advocated rebuilding the foundations of vision research, based on “evidence that the early steps of mammalian vision are more diverse and more interesting than usually imagined,

so that our understanding of the later stages is in trouble right from the start.”²⁵⁰. This thesis explored the non-primate perspective of RGCs as feature detectors, which led to the development of new hypotheses in areas where color vision has been “stuck”. Despite this success, it is worth emphasizing feature detection is not meant to be a one-size-fits-all perspective for retinal function. For example, one can imagine analyzing an S-cone from this perspective and reaching the misguided conclusion that it is a “419 nm detector”. As in the quote from Lettvin & colleagues (1959) at the beginning of this thesis, S-cones as 419 nm detectors “may be true, and yet, in a sense, be misleading.”²²⁷. It is striking and also a little concerning to see the significant role perspective plays in how we interpret visual neurons and circuits. If we hope to solve the mystery of vision, it will be important to continue actively exploring new perspectives.

Most of the work in this thesis emphasized how wavelength information can be used for purposes other than color perception. Here, I will first review the direct implications of this work for how we perceive color. Next, I will outline important unanswered questions in the areas addressed by this work: color vision models, ipRGCs and the diversity of retinal neurons. A few barriers to progress will be discussed and potential solutions will be proposed. Finally, I will discuss the advantages of connectomic reconstructions and outline ideas for how this technique could be used to accelerate progress in understanding rare retinal neurons.

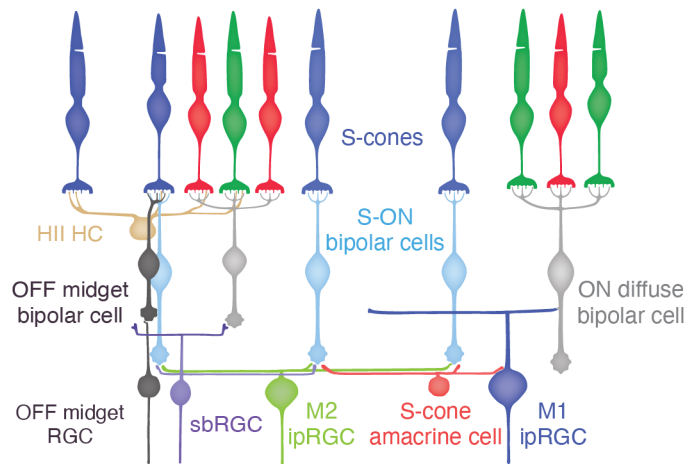


Figure 8.1 S-cone opponent circuits of the primate retina. The four RGCs all have S vs. L+M spectral tuning. Abbreviations: HC for horizontal cell, sbRGC for small bistratified RGC and ipRGC for intrinsically photosensitive RGC.

8.1 A “Missing” Dimension of Wavelength Information

In theory, potential cone-opponent neurons have just three possible ways to compare the activities of all three cone types: M+S vs. L, L+S vs. M and S vs. L+M. To review, trichromatic humans perceive four fundamental hues processed along two opponent axes^{163,177} and the cone inputs to these axes have been determined psychophysically: L+S vs. M for red-green and S+M vs. L for blue-yellow^{391,86}. The third possible cone-opponent pathway, S vs. L+M, matches the cone inputs to the small bistratified RGC, the most common trichromatic neuron in the early visual system. Yet S vs. L+M does not match the cone inputs to any of the fundamental hues (**Table 1**). Moreover, **Chapter 3** demonstrates conditions where large changes in S vs. L+M activity are not associated with any change in hue perception. From this perspective, whether or not the small bistratified RGC contributes S-cone input to cortical hue-encoding neurons downstream is important only from a historical standpoint and might miss the forest for the trees. If we took full advantage of trichromacy and extracted all of the wavelength information available at the level of the cone mosaic for hue perception, the S vs. L+M neurons would not just *contribute* to hue - instead they would *mediate* a distinct dimension of hue. With this extra dimension, we would not see four fundamental hues along two opponent axes but rather six fundamental hues along three opponent axes. In this regard, we are color-deficient relative to an optimal hue-encoding trichromat. For context, each opponent axis allows one to distinguish ~ 100 subtle gradations of hue²⁶⁶. With our two opponent axes, we can distinguish 10,000 distinct colors. Yet, this is only 1% of the total colors we would be able to see if we had access this third dimension of wavelength information.

This result is especially striking given that at least five RGC types carry this “missing” third dimension of wavelength information (**Figure 1.5** and **8.1**). The remainder of this thesis makes the case that the missing S vs. L+M information is not missing at all, but is instead being used for purposes other than hue perception. S-OFF midget RGCs may contribute wavelength information to a different aspect of perception: the edge detection that defines spatial vision and percepts of black and white. Meanwhile, M1 and M2 ipRGCs may contribute wavelength information to non-image-forming visual functions, such as circadian photoentrainment and the pupillary light reflex^{340,138}.

8.2 Color Vision Models

8.2.1 Which cells code for color?

A clear assumption of the work in this thesis is that if S vs. L+M and L vs. M neurons do not mediate hue perception, then there must be another set of neurons that do. The Neitz lab’s model, detailed in **Chapter 2.5**, proposes that a sparse subset of midget RGCs contribute to hue while the rest mediate spatial vision (**Figure 2.2F**). The idea that L vs. M midget RGCs serve only spatial vision has been proposed before^{311,44,324}, though the identity of the alternative hue-encoding RGCs has evolved over the past two decades to accommodate new insights from anatomy, physiology and psychophysics. However, this idea is generally met with a degree of skepticism that seems, to me, to be unwarranted and is worth discussing in the context of the future directions for this project.

The DeValois & DeValois (1993) multi-stage model described in **Chapter 2.4** is currently considered the ‘Standard Model’ (to borrow their terminology for an earlier two-stage model that was widely accepted at the time they proposed their model)⁸⁹. Yet most of the model is theoretical and lacks direct evidence for the proposed circuits in **Figure 2.2E**. The problem, as I see it, is that the multi-stage model is not falsifiable. The details of the model are vague enough to be reshaped each time conflicting evidence is revealed and a lack of evidence has never been considered grounds for rejection. For example, the model survived the discovery that the small bistratified RGCs do not project to the parvocellular LGN, as originally proposed²⁴⁸.

Current ideas about how color and spatial vision are “demultiplexed” from the outputs of L vs. M midget RGCs tend to fail in reliable ways⁹⁵. However, this is not considered problematic, perhaps in part due to our intuition that a cortex capable of things like consciousness *should* be able to figure out such a simple visual task. This is a difficult view to argue against, but it is worth noting that elementary vision is much harder than expected, as Marr (1982) noted in the introduction to his classic book on vision: “The first great revelation was that the problems are difficult. . . . The reason for this misperception is that we humans are ourselves so good at vision. . . . the idea that extracting edges and lines from images might be at all difficult simply did not occur to those who had not tried to do it”²⁴¹. For example, Kingdom & Mullen (1995) tried and concluded that “there is no unified scheme available to explain a separation of colour and luminance information in the visual system”¹⁸⁷. This is a rather large problem for the “Standard Model” on which very little progress

has been made.

Throughout the debate over spectral and spatial opponency in midget RGCs, there has been the assumption that the spectral information *must* be extracted - either to be used for color vision or to be discarded. The idea that the spectral information stays put and contributes to spatial vision, as proposed in **Chapter Two** and investigated in **Chapter Five**, is a unique aspect of this work and an insight reached by thinking of midget RGCs as feature detectors.

8.2.2 Stimuli

Most surveys of spectrally-opponent RGCs and LGN neurons found 5-10% of cells could be M vs. L+S or L vs. M+S^{82,350,96} and these results are at least acknowledged by many experts in the field^{336,186}. Many surveys that did not report L vs. M+S and M vs. L+S neurons also failed to find S-OFF midget RGCs in **Chapter 4**³⁴⁷ so their absence of evidence may not be evidence for absence (also a logical fallacy, *argumentum ad ignorantiam*³²¹). An important next step is to replicate the initial surveys of cone-opponent RGCs, then systematically investigate how the M vs. L+S and L vs. M+S neurons differ from more common cone-opponent RGCs and compare with the theoretical optimal hue-encoding receptive field discussed in **Chapter 2.3.2**. Recovering the morphology of these RGCs will be key. To be fair, the experiments above will only establish a correlation with hue perception, not causation. That being said, these neurons are a compelling alternative to the small bistratified RGCs, which, as discussed below, appear to be, at best, only weakly correlated with hue perception.

However, a significant concern is that, even if the same rare populations of M vs. L+S and L vs. M+S neurons were identified again, the result could be dismissed for the same reasons as the original studies. As shown in **Figure 1.4B**, only a small amount of S-cone input is necessary to align an L vs. M neuron with red-green and blue-yellow hue perception. Although the math behind silent substitution is elegant in theory^{114,368}, the implementation in biology is complex and accurate S-cone isolation is technically challenging²⁴⁷. As a result, the use of S-cone isolating stimuli to measure small S-cone inputs to midget RGCs above may not be compelling to skeptics.

A second concern is that, in light of the unexplained complexity of retinal processing in **Section 1.5**, are we certain the classic battery of cone-isolating spots, annuli and drifting gratings is going to be effective for every different type of cone-opponent neuron? The rarity of most uncharacterized

cone-opponent RGCs certainly contributes to their absence in past studies; however, the stimuli used could be another contributing factor. An unexpected result of the S-OFF midget RGC recordings in **Chapter 4** was that their inhibitory surround receptive field was generally stronger than in L/M-cone midget RGCs. The weak responses to full-field stimuli in **Figure 4.4A** could explain their absence in many surveys using full-field stimuli³⁴⁷. Are there other aspects of the standard stimuli that might bias against rarer cone-opponent neurons? This is worth exploring because, at least in my experience recording from uncommon RGCs, artificial stimuli that *should* work, sometimes do not.

What do the relative strengths of the L-, M- and S-cone inputs mean for a neuron's role in vision? They provide insight into the wavelength information that is present in an RGC's response, which might have been a sufficient characterization if RGCs were passively encoding information, as in **Figure 1.3**. However, if retinal circuits play a more specific role in downstream functions, whether or not wavelength information is present in a neuron's output should be the first question, not the last. It took most of my graduate career to fully appreciate that linear cone-isolating stimuli are, in a sense, mapping a neuron's input, not its function nor its role in vision. For example, consider the beautiful cone maps obtained through spatiotemporal noise stimuli by Field & colleagues (2010)¹²¹. These artificial stimuli are essential for testing specific mechanistic hypotheses, but may be very limited as a measure of a neuron's role in vision. A set of stimuli geared more towards understanding downstream functions or how different receptive field dimensions integrate would add to the insights learned from existing stimuli.

That being said, it is easy to criticize artificial stimuli, but much harder to come up with useful alternatives³¹⁷, especially to survey of diverse set of neurons with unknown properties. My opinion is that we do not complex new stimuli or new ways of exploring the same cone-opponent space³⁴⁷, but would instead benefit most from revisiting stimuli that define a neuron by its responses to the physical characteristics of light (i.e. wavelength and intensity). Cone-isolating stimuli have been in fashion for decades and with good reason - the stimuli can be used with conventional video displays³² and the results are more straightforward to organize and quantify⁹⁶. By comparison, working with many monochromatic lights is a technical challenge and also harder to interpret and standardize. Fortunately, the new stimulator used in **Chapter 3** can enable measurements of spectral sensitivity functions with an unprecedented level of spatial, spectral and temporal control.

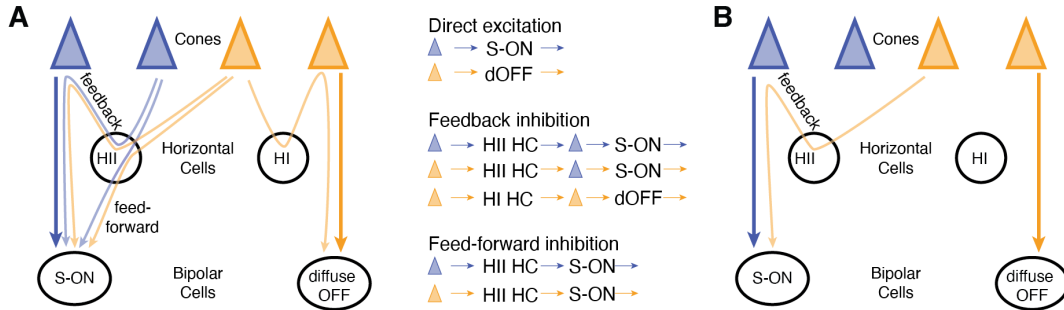


Figure 8.2 Outer retina circuitry for the small bistratified RGC as revealed by our connectomic reconstructions (**A**) and as investigated by electrophysiology (**B**).

The measurement of spectral sensitivity functions would provide a complementary approach to cone-isolating stimuli and new perspectives on how wavelength information is processed by cone-opponent RGCs. The value of this approach is clear from the continued relevance of classic psychophysical measurements of spectral sensitivity curves. Examples include the saturation discrimination function in **Chapter 5**, the wavelength discrimination function and the opponent response functions. These measurements are elegant, straightforward and have refreshingly few of assumptions compared to the color spaces and silent substitution used in modern psychophysics experiments. Moreover, as discussed in **Chapter 5**, the underlying neural substrates for most are still unclear and there is much to learn from investigating them.

8.2.3 *If not blue-yellow, what does the small bistratified RGC do?*

The results in **Chapter 3** may be surprising, given that the small bistratified RGC was long thought to be the substrate for blue-yellow color vision⁷¹. The S-ON and LM-OFF subunits were originally predicted to perfectly overlap in space and cancel in response to white light, creating a “pure color cell”. In practice, however, small bistratified RGCs do respond well to achromatic, spatially structured stimuli^{350,123,349}. Moreover, the “blue-ON” LGN neurons receiving small bistratified RGC input have surprising properties inconsistent with blue-yellow hue perception, such as binocular integration and direction selectivity^{400,350}.

Perhaps spectral opponency is not *the* defining feature of the small bistratified RGC, but just the most defining feature we know of thus far. Past studies have largely focused on their role in color vision and particularly whether they have ‘Type II’ receptive fields (see **Chapter 2.3.2**)^{384,71,63,122,111}. A new set of experiments exploring the small bistratified RGC’s responses to stimuli unrelated to

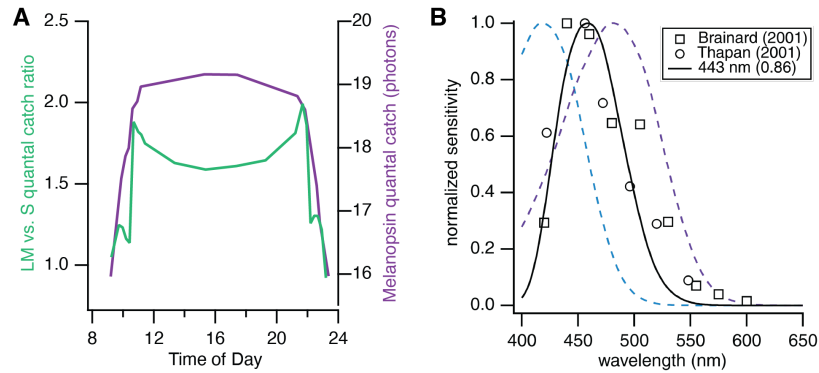


Figure 8.3 (A) Melanopsin quantal catch (purple) and LM- vs. S-cone quantal catch (green) throughout the day. Adapted from Pauers et al (2012)²⁸⁵. (B) The spectral sensitivity of melatonin suppression. Data was obtained from Brainard et al (2001) and Thapan (2001) and fit to a standard photopigment template^{33,354,50}. The spectral sensitivities of melanopsin and S-opsin are shown in purple and blue dashed lines, respectively.

hue perception could yield new insights. One possible approach is to explore responses to different “achromatic” stimuli. In most studies, achromatic stimuli are defined as having equal L-, M- and S-cone activations^{350,63}. This makes perfect sense for testing the relative weights of S- and L/M-cone input, which are predicted to be equal for a ‘Type II’ receptive field. However, different cone weights are needed to resemble perceptually “achromatic” or white light²⁰⁴ and this discrepancy may be critical, given the small bistratified RGC’s strong cone opponency.

Another area to investigate is whether small bistratified RGCs could contribute to some type of motion detection. In an unpublished set of experiments, I confirmed the small bistratified RGCs do show some direction selectivity³⁴⁹, spatial opponency and interesting responses to various motion stimuli. Moreover, their density increases dramatically in the peripheral retina, a region tailored to motion rather than color perception. How exactly the small bistratified RGC’s robust spectral tuning would contribute to motion is not immediately clear to me, but would be an interesting area to explore. A final possibility is that there is no feature and the next iteration of coding hypotheses will provide a better perspective on the small bistratified RGCs’ odd mixture of spectral, spatial and directional information.

8.3 Intrinsically Photosensitive Retinal Ganglion Cells

Primate RGC receptive fields are classically described in relation to specific aspects of visual perception (e.g. “red-green” midget RGCs). Because we understand the ipRGCs’ function arguably better than any other primate RGC, they provide a unique opportunity to explore the limitations

of the above approach for understanding a neuron’s role in visual perception and behavior.

M1 ipRGCs integrate responses from rods, cones and melanopsin to signal intensity over a wide range of light levels^{208,252}, however, we know that calling them “intensity detectors” is not the most accurate description of their role in vision. Moreover, their receptive fields are well-suited to encode changes in wavelength, but calling ipRGCs “wavelength detectors” is incomplete as well. Instead, these are just different aspects of the ipRGC’s receptive field that serve the neuron’s downstream function: detecting the time of day, or more precisely the location of the sun in the sky, by encoding the features of the visual scene that change with solar angle^{386,338}. This point emphasizes two important themes central to this thesis. First, we can learn more from other species than previously thought. This essential insight into the ipRGCs’ role in vision was learned from the mouse retina, and, fortunately, M1 and M2 ipRGC anatomy, physiology and function appears largely conserved^{22,101,263}. Second, feature detection works best when one thinks of features in terms of downstream functions rather than stimulus attributes.

8.3.1 The contribution of S-cones to circadian photoentrainment

An unexpected finding in this thesis is that M1 and M2 ipRGCs receive opposite S-cone input; the S-cone amacrine cell input to M1 ipRGCs is inhibitory, while the S-ON bipolar cell input to M2 ipRGCs is excitatory. In the past, the wide-ranging effects of short-wavelength light on human health and disease were largely attributed to melanopsin. **Chapters 6** and **7** establish a more complex set of underlying mechanisms which could involve different combinations of S-cone ON, S-cone OFF and melanopsin-mediated responses. These findings raise exciting new avenues for future research about the role of S-cones in non-image-forming visual functions, particularly circadian photoentrainment.

An important first question to address is the extent to which each ipRGC subtype contributes to circadian photoentrainment. It is clear that only some mammalian M1 and M2 ipRGCs project to the suprachiasmatic nucleus and directly control circadian photoentrainment^{55,228,315}. Do the ipRGCs in **Chapter 6** and **7** contribute to circadian photoentrainment? While it will be essential to obtain direct evidence, several lines of evidence strongly support S-cone and cone-opponent contributions to circadian photoentrainment. As shown in **Figure 8.3A**, the peaks in LM vs. S-cone contrast at sunrise and sunset provides a robust, temporally-precise cue for time of day^{338,386}.

Accordingly, the use of cone opponency in circadian photoentrainment is found in a diverse range of species and may be evolutionarily conserved^{341,258}. Second, the spectral sensitivity of melatonin suppression cannot be explained by melanopsin alone^{33,354}. As shown in **Figure 8.3B**, the best-fitting spectral sensitivity curve peaks at 443 nm, suggesting the involvement of S-cones³⁰⁴.

A second future direction is a physiological investigation of S-cone amacrine cell-mediated inhibition to ipRGCs. There are two possible, non-mutually exclusive for how S-cone amacrine cell inhibition contributes to encoding sunrise and sunset. The global changes in S vs. L+M cone contrast shown in **Figure 8.3A** are well-documented^{285,338,386} but too slow to drive transient spiking in circuits built to signal spatiotemporal contrast. However, there is considerable local spectral contrast in natural scenes at sunrise and sunset that could drive a spectrally-opponent neuron as the eye makes saccades. The S-cone amacrine cell could help drive spiking in ipRGCs through transient disinhibition. Second, the S-cone amacrine cell could operate by provide tonic inhibition to ipRGCs tracking the gradual changes in the color of the sky shown in **Figure 8.3A**. Photoreceptors have transient responses as well as a sustained steady-state response that reflects the average light level²⁷³. These tonic and phasic response components can be used by downstream circuits to encode the contrast and the mean, respectively^{275,142}. Because S-cones' output is spectrally-opponent²⁷⁸, their steady-state response will reflect the average spectral distribution (i.e. the mean S-cone quantal catch relative to that in L/M-cones) and follow the gradual changes in S vs. L+M cone activity shown in **Figure 8.3A**. The steady-state response is largely filtered out from the responses of most RGCs, but is inherited by bipolar cells¹⁰⁷ and may be present in the S-cone amacrine cell's output. If so, at sunrise and sunset when S-cone activity is lowest relative to L/M-cone activity, one could predict a decrease in tonic inhibition from the S-cone amacrine cell and a depolarization of the ipRGC. The increased relative S-cone activity at other times would hyperpolarize the ipRGCs and limit their spiking so that circadian rhythms are not constantly susceptible to spectral and spatial contrast. Mammalian M1 ipRGCs are reported to receive significant tonic inhibition³⁸⁸. Future experiments are needed to confirm this in primate ipRGCs and measure the spectral tuning of the tonic inhibition.

A word of caution is that the interpretations above account for just one aspect of the ipRGC's receptive field and could fall into the same trap as the various "optimal" midgenet RGCs receptive fields in **Chapter 2.3.3**. It is fair to say that we still know very little about the S-OFF responses

in ipRGCs and even less about how the S-cone input might integrate with other cone, rod and melanopsin-mediated responses. Future experiments will need to not only characterize the different response properties of each subtype individually, but also investigate how they interact.

8.4 Understanding Rare Retinal Neurons

The work in this thesis provides a substantial advance towards a complete picture of S-cone circuits in the primate retina. However, we are still very far from a full understanding of retinal diversity. That most of the >20 RGC types and >40 amacrine cells have no known function indicates that we still may only understand a small fraction of the visual processing occurring within the primate retina. The need for information on rare retinal neurons is matched by the difficulty of deducing their function from electrophysiology alone. If each rare RGC type is a feature detector, then the question is what feature does each rare primate RGC compute? The challenge is identifying the feature, especially when rare cells are infrequently encountered, recording time is limited and stimuli must be planned in advance. The most intractable aspect may be that the neural circuits between the photoreceptors and an unknown RGC's spike output are a black box. Connectomic reconstruction with serial electron microscopy can fill this gap in knowledge and is particularly effective in situations where there is little initial physiological data, as in **Chapter 6** and **7**. Even with well-studied neurons like the small bistratified RGC, reconstruction of upstream neurons and synaptic inputs can inform new hypotheses and reveal unstudied circuit elements (**Figure 8.2**).

Retinal anatomy and physiology are often treated as two distinct domains, with the physiological recordings of a neuron's output generally regarded the ground truth¹⁴. However, this view seems poised to change⁹³. First, recent technical advances have made connectomics more manageable than ever⁹³ and capable of addressing previously intractable research questions^{100,35}. Second, it is increasingly clear that receptive fields revealed by electrophysiology are not static, but are instead dynamic and flexible with different circuit mechanisms operating under different conditions^{309,383}. Thus, recordings may not reveal a single truth but rather a single snapshot of a neuron's response properties that depend on retinal eccentricity³³², recording technique^{123,63,215}, background light levels^{107,122} and stimulus choice. Connectomics can guide physiology by providing a blueprint of the different circuits available to a neuron that could be engaged under different experimental conditions. For example, compare the map of the small bistratified RGC's outer retinal circuits

in **Figure 8.2** revealed by connectomics to those investigated by past physiology experiments. In turn, physiology is needed to establish key biophysical properties of each neuron, as well as the operating range and integration of circuit elements identified anatomically.

A natural future direction is to bridge these anatomical and physiological approaches through the development of biophysical models. Neuronal morphology defines passive electrical properties and, given the highly stratified nature of the inner retina, constrains the identity, location and integration of excitatory and inhibitory synaptic inputs^{190,109}. These features could be explored by converting serial EM reconstructions to detailed biophysical models or by creating simplified models that capture the salient aspects of a neuron’s morphology and synapse distribution revealed by the reconstructions^{117,143}. The reconstructions in this thesis were annotated to optimize representation by cylinders so the underlying data will translate well to models relying on cable theory³⁰².

This approach would establish feedback loop between connectomics and electrophysiology by enabling *in silico* experiments testing specific hypotheses about how different synaptic inputs shape a neuron’s responses. For example, what is the functional relevance of the location of S-cone amacrine cell inhibition to ipRGCs¹³³? M1 ipRGC dendrites receive hundreds of synapses yet these inputs must travel through the dense S-cone amacrine cell input to reach the axon (**Figure 6.3**). Although hindsight is always 20/20, it seems reasonable that, for example, the starburst amacrine cell’s compartmentalized dendrites¹¹⁵ could have been predicted from simplified biophysical models while their tuning to centrifugal motion could have been predicted from the distribution of input and output synapses along each dendrite^{118,367}. Finally, biophysical models may provide a helpful alternative to the standard linear-nonlinear models³⁰⁵ in situations where the details of a neuron’s dendritic field cannot be abstracted¹⁶⁴. By combining anatomy and physiology in this way, the function of rare primate RGCs may be easier to investigate.

8.5 Concluding Remarks

This thesis advanced our understanding of S-cone opponent retinal circuits and how wavelength information can be used by the visual system. This work also revealed explored the shortcomings of existing models of primate vision. By investigating new perspectives and techniques, this thesis identified promising directions forward towards a clearer picture of the retinal substrates of visual perception and behavior.

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