Functional Diversity of Retinal Ganglion Cells in the Rat

by

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Neurobiology in the Graduate School of Duke University

2017
ABSTRACT

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Abstract

One of the central problems in neuroscience is that there is a lack of understanding of the diversity and functions of cell types in the brain. Even in brain areas that have been studied extensively, such as the retina, much remains to be learned about the diversity and functions of cell types. Morphological, functional and genetic studies have yet to converge on a consistent picture of cell type diversity in the retina, because the field lacks a standardized approach to classify cell types. A systematic classification approach is essential to provide an unambiguous appreciation of cell type diversity, and a better understanding of the organization and function of the retina. In the first portion of this dissertation, we present a novel approach that classifies retinal ganglion cells (RGCs) in a quantitative, verifiable and reproducible manner. We utilize diverse visual stimuli and a multi-electrode array, to record simultaneously from multiple RGCs, and show that there are at least 13 RGC types with distinct functional properties. In the second portion of the dissertation, we present a quantitative determination and comparison of the spatiotemporal receptive field (RF) structures and neural coding properties across these RGC types. Determining the RF structure of RGC types is important, because it constrains the computations performed by retinal circuits and identifies the signals available to retinal recipient areas. We find that RGC types exhibit functional asymmetries in terms of their RF size, temporal integration, and response nonlinearities. We also show that no RGC types exhibited RFs that were strictly
independent in space and time. These results provide several new insights into the computations performed in the rodent retina, and highlight the importance of understanding cell type diversity to further our understanding of how the retina works and the role it plays in visual processing.
Dedication

To my loving parents Prabha Mummy and Ravi Appa

To my beloved husband Hari

To my doting in-laws Sasikala Amma and Sivakumar Appa

To my affectionate brothers: Sunand and Sriram

To my endearing grandmothers: Vijaya and Leela Thaathi

And to the One who is my heart: BSSSB

Jai Sai Ram.
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1. Introduction

One of the central problems in neuroscience is that of determining the diversity and functions of cell types in the brain (Cook, 1998; Masland, 2004; Wichterle, et al., 2013; Brain Initiative Working Group, 2014; Seung & Sumbul, 2014; Poulin, et al., 2016). This is a basic issue, but is fundamental to our understanding of how the brain works. Different cell types perform different functions, and it is only through the discovery and definition of cell types that one can start to piece together the functions performed by the brain (Poulin, et al., 2016). Determining the function and diversity of cell types is useful not only to understand the function of the brain; it also allows the development of better neural regenerative therapies that target specific cell types, enables the genetic manipulation of cell types; and facilitates the building of better computational models that reflect the computations performed by distinct cell types (Wichterle, et al., 2013; Seung & Sumbul, 2014).

In this thesis, we focus on studying cell type diversity in the retina. We target the retina because it is an extensively studied part of the central nervous system, more readily accessible than other brain areas, and has a clearly defined function: vision (He, et al., 2003; Masland, 2004). Specifically, we determine the diversity and function of a class of cells known as the retinal ganglion cells (RGCs). RGCs are the final output neurons of the retina that send signals to the brain. RGCs have been a major focus of cell type diversity studies (Cajal, 1893; Masland, 2001; Wassle, 2004; Sanes & Masland, 2015).
since they are the only cells that convey information about the visual world to the rest of the brain. Even though RGCs have been investigated for over a century (Cajal, 1893; Seung & Sumbul, 2014), studies have yet to converge on the diversity and functions of RGC types. In the sections below, I review the anatomical and functional approaches that have attempted to classify RGCs and discuss the importance of understanding RGC type diversity and function.

**1.1 Definition of a retinal ganglion cell type**

Before examining the different approaches that classified RGC types, it is first essential to define a type. A RGC type is defined as a group of RGCs with the same anatomical, functional and molecular properties (Cook, 1998; Masland, 2004; Seung & Sumbul, 2014). One example of an RGC type is the alpha cell. The alpha cell has a large soma, brisk transient responses to visual stimuli, and can be identified using neurofibrillary staining methods. A distinctive attribute of the alpha cells is that they arrange their dendrites in a territorial way, such that there is minimal overlap between the dendritic fields of adjacent cells (Figure 1). Their cell bodies are also regularly spaced (Wassle, et al., 1981; Wassle, 2004). This non-random distribution of dendritic fields and cell bodies across the retina is known as “tiling”, and allows the alpha cells to convey information about a visual feature over the entire visual field. Tiling provides the validation that cells with the same anatomical, functional and molecular properties constitute a pure cell type rather than a mixture, and should not be subdivided any
further; i.e. that a cell type is irreducible (Cook, 1998; Cook, 2003; Field & Chichilnisky, 2007; Masland, 2012; Seung & Sumbul, 2014).

Figure 1: The dendrites and somas of the ON alpha cells tile the retina uniformly and independently of each other (Wassle 2004).

Other RGC types also exhibit dendritic tiling (beta cells: Peichl & Wassle, 1979; Wassle, et al., 1981; direction selective ganglion cells: Amthor & Oyster, 1995). Moreover, RGCs are not the only cells in the retina that exhibit dendritic tiling. Starburst amacrine cells (AC), bipolar cells (BC), horizontal cells (HC) and cone photoreceptors (PR) all exhibit anatomical dendritic tiling (Wassle & Riemann, 1978; Wassle, et al., 1981; Vaney, 1994; Keeley, et al., 2007; Wassle, et al., 2009). The anatomical tiling of RGC types has also been shown to translate functionally. This is because the dendritic field size determines the receptive field size, or the region in visual space that RGCs respond to (Yang & Masland, 1994). Cells of the same type have receptive fields that sample visual space in such a way that the entire visual field is sampled with minimal overlap and no blind spots (Devries & Baylor, 1997; Shlens, et al., 2006; Field, et al., 2007; Petrusca, et al., 2007; Gauthier, et al., 2009; Anishchenko, et al., 2010; Sher & DeVries, 2012). Receptive field tiling has been demonstrated across 11 RGC types in the rabbit (Devries & Baylor,
1997), 5 RGC types in the primate (Chichilnisky & Kalmar 2002, Field, et al., 2007; Gauthier, et al., 2009; Jepson, et al., 2013) and 4 RGC types in the rat (Anishchenko, et al., 2010). Uniform anatomical and functional tiling is therefore regarded as a fundamental principle of retinal organization. Therefore, when characterizing the diversity of RGC types, it is critical to validate if a RGC type that has distinct properties is an irreducible type by verifying uniform sampling of visual or anatomical space.

1.2 Classifying and characterizing the diversity of RGC types

Judging from the studies above, it might seem like the retina is a well-studied part of the nervous system and the general principles of retinal organization, such as tiling, are well established. Though this is certainly the case, much remains to be understood about the diversity of RGCs and the aspects of visual information they signal to the brain. This is because even though various studies have attempted to classify RGCs and study the diversity of RGC types in terms of their anatomy or physiology, there is still no consensus on the diversity of types and the computations carried out by each type. Studies have identified anywhere between 5 - 30 RGC types (Devries & Baylor, 1997; Huxlin & Goodchild, 1997; Rockhill, et al., 2002; Sun, et al., 2002; Carcieri, et al., 2003; Badea & Nathans, 2004; Dacey, 2004; Coombs, et al., 2006; Volgyi, et al., 2009; Farrow & Masland, 2011; Baden, et al., 2016). Additionally, other than a few well-studied RGC types (alpha and beta cells: Enroth-Cugell & Robson, 1966; Boycott & Wassle, 1974; Cleland & Levick, 1974; DS cells: Barlow & Levick, 1965; Yang &
Masland, 1994; Amthor & Oyster, 1995), many RGC types have not been reproducibly identified across multiple preparations.

According to a recently published article in Nature (Baker, 2016), the failure to reproduce results across experiments is actually a bigger scientific problem: more than 50% of researchers have failed to reproduce their own experiments and more than 70% have tried and failed to reproduce another scientist’s experiments. Thus, for the retinal field to advance and reach a consensus on the RGC types and the computations performed in the retina, it is critical to develop a classification method that ensures that identified cell types are cross-validated and reproducible across experiments. Additionally, the cell types need to be verified by checking for anatomical or functional tiling, and precisely identified using easy-to-measure response properties, that can be retraced by other scientists to extract and study the same cell types (Carcieri, et al., 2003). Below, I discuss in detail the anatomical and physiological studies that have attempted to classify RGC types, examine some of their limitations, and identify how that led to some of the ideas behind developing a novel classification method to characterize the diversity of RGC types.

1.2.1 Anatomical classifications of RGC types

Numerous anatomical studies have tried to determine the diversity of RGC types in the mammalian retina. The first anatomical studies of RGC types were performed by Cajal using Golgi staining on ox, dog and frog retinas. 10-15 varieties of RGCs in each
species were identified based on their dendritic tree size, morphology, and stratification (Cajal, 1892). Cajal also deemed that RGC types were remarkably similar across species, which has been confirmed by recent anatomical and functional studies (Peichl, 1991; Kolb, 2001; Sun, et al., 2002a; Sun, et al., 2002b; Wong, et al., 2012). This was followed by Polyak who classified RGCs in primate into 5 categories using their dendritic field and cell body sizes (Polyak, 1941; Polyak, 1957; Wandell, 1995; Kolb, 2001). The next set of breakthroughs happened in the cat retina, where Boycott and Wassle established that there were 3 morphological RGC types: alpha, beta and gamma (Boycott & Wassle, 1974). These 3 types were shown to correlate with the 3 morphological types identified in the primate (Y, W and X; Fukuda & Stone, 1974; Leventhal, et al., 1981; Shapley & Perry, 1986) and the rat (Boycott & Wassle, 1974; Fukuda, 1977; Heine & Passaglia, 2011; Wong, et al., 2012).

The development of transgenic mice, immunohistochemistry, and new imaging techniques, allowed larger numbers of RGCs to be surveyed, and the number of anatomical RGC types continued to change. Advances in retrograde labeling and Golgi staining of whole mount retinas increased the number of RGC types in the cat to 23 (Kolb, et al., 1981; Kolb, et al., 1992) and in the primate to 13 (Dacey, et al., 2003). Carbocyanine dye (Dil) and Neurobiotin labeling established 10 RGC types in the rat (Huxlin & Goodchild, 1997), a study using transgenic mice with an alkaline phosphatase reporter identified 9 RGC types in the mouse (Badea & Nathans, 2004), DiOlistic
labeling of RGCs distinguished 16 similar RGC types in the rat and mouse in one paper (Sun, et al., 2002a; Sun, et al., 2002b), and 14 RGC types in the mouse in another paper (Coombs, et al., 2006).

The anatomical approach has had difficulty achieving consensus, due to the following reasons: a) Most anatomical studies have been unable to verify the irreducibility of each cell type, by checking for the presence of dendritic tiling. This is due to the limited availability of markers that label specific cell types (Seung & Sumbul, 2014; Sanes & Masland, 2015). Therefore, there is always a degree of ambiguity about the classified anatomical cell types, because it is possible that they are variations of the same cell type across eccentricity or across preparations. b) All morphological surveys have combined data across experimental preparations from different retinas, and not cross-validated the cell types found in one preparation across multiple other preparations. Cross-validation is essential to figure out how well a classification scheme will generalize to new data. This in turn, will allow new studies to be able to reproduce the results from previous studies, and add any new discoveries to the existing classification database. c) There is no standardized method to anatomically classify RGCs. Different studies have used different techniques to visualize the somas and dendrites of RGCs, studies have been either qualitative or quantitative in their grouping of RGCs, and quantitative studies have made use of various clustering techniques to group cells (Badea & Nathans, 2004; Kong, et al., 2005; Sumbul, et al., 2014). Though this has
resulted in the development of many novel techniques, it can also lead to uncertainty in establishing the diversity of cell types, because each study determines its own set of cell types. Hence, it is clear that in order to understand the diversity of RGC types and how each type contributes to visual processing, the field needs a standardized method that can establish the same basic number of types across preparations, and validate each type by checking for the presence of tiling. Once a basic framework is laid and a consensus is achieved, new studies can build on that framework to generate a complete understanding of cell type diversity and visual processing in the retina.

1.2.2 Physiological classification of RGC types

1.2.2.1 Early history of physiological RGC types

Functional studies have also tried to determine the types of RGCs, by classifying RGCs according to their light response properties. This in turn has resulted in an increased appreciation for the computations performed, and features of visual scene extracted at the level of the retina. Hartline (1938) made the first physiological description of how RGCs parcel the visual scene when he found that cells in the frog retina responded to either light increments (ON), decrements (OFF) or both (ON-OFF) (Hartline, 1938). These findings were refined by Kuffler, who noted that OFF cells in the cat retina were stimulated by a light offset in the center of their receptive field, but suppressed by a light offset in the periphery. This led to the concept of a receptive field and center-surround antagonism. The receptive field (RF) represents the spatial and
temporal features of visual scenes that are transmitted to the brain and summarizes the computation performed by a RGC (Barlow, 1953; Kuffler, 1953; Gollisch & Meister, 2010). The RF of a cell can be divided into two regions: the center and surround, which interact antagonistically with each other, for e.g., an ON-center/OFF-surround cell and an OFF-center/ON-surround cell.

A further subdivision of ON and OFF cell types in the cat retina came about, when Enroth-Cugell and Robson (Enroth-Cugell & Robson, 1966) discovered that cells could be divided into those that summed spatial inputs linearly (X cells), or non-linearly (Y cells) when presented with contrast-reversing gratings. The four functional types in the cat were found to correlate with unique morphological types, leading to the conclusion that RGCs with a unique structure carried out a unique function (Cleland, et al., 1971; Boycott & Wassle, 1974; Cleland, et al., 1975; Levick, 1975). X cells had small cell bodies and dense dendritic fields; Y cells had large cell bodies and sparse dendritic fields; and ON and OFF cells stratified at different layers in the inner plexiform layer (IPL) (Nelson, et al., 1978). These 4 cell types (ON and OFF X and Y) were also found consistently across multiple species: ON and OFF parasol and midget cells in the primate (Shapley & Perry, 1986), and ON and OFF alpha and beta cells in the mouse (Peichl, 1991; Sun, et al., 2002b), rat (Heine & Passaglia, 2011; Wong, et al., 2012), and ferret (Vitek, et al., 1985; Isayama, et al., 2009).
Thus the four basic cell types in the retina were established, and along with it the evidence that each RGC type had a distinct structure, function, molecular make-up and tiled the retina. The strength of these early studies lay in the fact that each cell type could be reproducibly identified in new studies, and even across species. This was because each type was defined in terms of its anatomy, physiology, molecular markers, tiling, and its projections to higher visual areas (ventral/dorsal stream; M and P pathway). Thus, the computations performed at the level of retina, as well as how each cell type guided visual perception started to become clear.

1.2.2.2 **RGC types with more complex response properties**

The development of electrophysiological techniques and the use of more complex stimuli led to a more detailed quantification of response properties of existing cell types, as well as the discovery of new cell types with more complex features. Direction selective cells, which responded to motion in one direction, were found in the rabbit retina (Barlow & Hill, 1963). Blue-On cells were discovered in the primate (Dacey & Lee, 1994; Dacey & Packer, 2003), local edge detectors in the rabbit (Levick, 1967), and uniformity detectors in the rabbit and cat (Stone & Hoffmann, 1972; Troy, et al., 1989; Taliby, et al., 2007). More recently, intrinsically photosensitive ganglion cells (ipRGCs), which are responsible for circadian entrainment and the pupillary light reflex, have been found in the mouse (Berson, 2003).
These results indicated that the operation performed by at least some RGCs was not just a simple spatiotemporal filter applied to a visual scene. RGCs carried out complex computations such as the detection of motion, and filtered visual information into more than four subcategories. It was thought that the retina partitioned visual information into at least 20 parallel channels, since morphological studies had identified up to 23 types. However, since all of these studies were carried out using single cell recordings, each study relied on a few example cells to define a class. Moreover, the criteria used to classify cells were rarely quantitative or and did not check for the presence of tiling. The functions performed by more than half of the 20 RGC types were also still unclear.

1.2.2.3 Multi-electrode arrays and large-scale recordings of RGCs

To elucidate the functions of more RGC types, 64-channel multi electrode arrays (MEA) were utilized. The MEAs allowed recording simultaneously from many RGCs for long periods of time (Meister, et al., 1994) and the quantitative classification of multiple RGC types according to variations in their response properties. Though this approach increased the number of cell types found, there was still a lack of consensus across studies on the diversity and functions of RGC types. One reason for this was because each study took a very different approach to classifying RGC types. For example, Devries and Baylor observed qualitatively that there were 8 types of RGCs in the rabbit that uniformly sampled visual space (Devries & Baylor, 1997). Carcieri et al. formalized
the classification of responses to receptive field illumination using a statistical bootstrapping method and found 5 types of RGCs in the mouse (Carcieri, et al., 2003). Farrow and Masland found that unsupervised clustering in the mouse yielded 12 distinct RGC types (Farrow & Masland, 2011). Segev et al. discovered that RGCs in the salamander formed a continuum and could not be separated into distinct types (Segev, et al., 2006). A recent study by Baden et al. utilizing two-photon calcium imaging and unsupervised clustering found up to 32 RGC types in the mouse, which was considerably more than the 20 or so estimated from anatomical studies (Baden, et al., 2016). Hence, studies were unable to provide a unified picture of the diversity of RGC types.

1.2.2.4 Convergence of RGC type diversity

Studies were unable to converge on the diversity and functions of RGC types due to the following reasons:

a) Cross-validation across experimental preparations. All functional surveys combined data across multiple preparations from different retinas. The cell types found in one preparation were not cross-validated across multiple other preparations. As mentioned above, cross-validation is essential, to figure out how well classified RGC types will generalize to new data. This in turn, will allow new studies to be able to reproduce the results from previous studies, and add any new discoveries to the existing classification database.
b) Verification of cell types. Most functional studies have been unable to verify the irreducibility of each cell type by checking for the presence of RF tiling. This is because limitations in recording techniques do not allow cell types to be verified across preparations by checking if all cells within a type tile space to form a mosaic. Therefore, there is always a degree of ambiguity about the functionally classified cell types, and it is possible that they are variations of the same cell type across preparations.

c) Classification of cell types using well-defined response features. Functional studies have used either qualitative or quantitative methods to classify cells. Moreover, quantitative studies select random features and apply automatic clustering algorithms that do not establish rigorous boundaries between cell types. Selecting features that precisely define cell types is essential, to identify functional properties that are unique to a cell type. This will allow new studies to retrace previous studies and classify RGC types by using the same set of features and response properties. Any new findings put forth by future studies concerning existing cell types can then be added to the list of functions performed by these types to enhance our understanding of individual RGC types and how they contribute to visual processing.

As discussed above, the development of novel imaging methods (Baden, et al., 2016) and large-scale multi-electrode arrays (Litke, et al., 2004) has resulted in the ability to monitor the activity of hundreds of RGCs simultaneously. This opens up the possibility of determining the diversity and functions of all RGC types. Hence, it is
important to develop a rigorous and standardized classification scheme that allows RGC types to be classified in a reproducible, verifiable and quantifiable manner. This will enable the field to reach a consensus on the functions of diverse RGC types, which will in turn improve our understanding of how the retina works and contributes to visual processing.

1.2.3 Importance of understanding cell type diversity and function in the retina

Understanding cell type diversity in the retina improves our understanding of visual processing in the following ways. First, it informs us about the diversity of functions performed by the retina. By studying cell type diversity, one can determine if particular a color, motion or orientation is processed at the level of the retina. This will allow us to gain an appreciation for the aspects of the visual scene that are extracted in the retina. Once the features extracted by the retina are known, it will be possible to generate hypotheses about how the presynaptic circuitry aids in extracting these features, and how this information is integrated and processed by downstream areas.

For example, it was possible to generate various hypotheses for how starburst amacrine cells gave rise to direction selectivity (DS) (Vaney, et al., 1989; Yoshida, et al., 2001; Fried, et al., 2002; Briggman, et al., 2011; Taylor & Smith, 2012), only when it was known that that DS was a feature extracted by RGCs (Barlow, et al., 1964; Barlow & Levick, 1965). Additionally, examining whether the features extracted by the retina generalize across
species can provide insights into whether this is a constraint imposed by the visual scene or due to the ecological niche of the animal.

Second, by understanding the computations and functions performed in the retina, we can build better computational models of neurons. For example, some cell types might perform simpler functions and be well described by simple linear models, while other cell types might perform complex functions and be better described by complex nonlinear models. These computational models can provide insight into the presynaptic mechanisms which give rise to the computations. For example, the motion computation performed by DS-RGCs could be described by the Reichardt-Hassenstain model (Borst, 2000), and led to the insight that a temporal delay might result in preference for motion in one direction over another. This implied that the time delay between excitation from bipolar cells and inhibition from starburst amacrine cells might give rise to direction selectivity (Torre & Poggio, 1978; Taylor & Vaney, 2002). Hence, understanding the diversity of computations performed by RGC types and building better models to describe these computations could provide a better intuition for how presynaptic circuitry such as BC types and AC types might give rise to the computation. This will improve our understanding, not just of the functions of RGCs, but of the function of retinal circuitry as a whole.

Third, if we know the functions of cell types and what downstream areas they project to, we can better understand the signals and information conveyed to
downstream visual areas. From this, we can gain a better appreciation for how these signals are integrated together by cells in downstream visual areas to refine the visual image and give rise to visual perception and behavior. For instance, orientation selectivity is thought to arise in the visual cortex through both feedforward and feedback signals, however, it is unclear how much of the information comes from feedforward signals and what these signals encode (Hubel & Wiesel, 1962; Scholl, et al., 2013; Zhao, et al., 2013; Dhande, et al., 2015). Determining if the feedforward signals entering the cortex are conveying information about specific orientations, or the strength of orientation tuning can better inform how the cortex modifies these signals to give rise to our perception of orientations in the visual world. This could eventually provide the link between the processing of visual information in the retina and how this gives rise to visual perception and behavior in the animal.

Fourth, a thorough understanding of cell types in the retina and their unique anatomical, functional and molecular make-up aids in targeting and genetically manipulating specific cell types. The ability to genetically manipulate specific cell types allows them to be studied in more detail through visualization with different reporters or optogenetics (Sanes & Masland, 2015). It also enables specific cell types to be silenced and their contribution to visual processing as a whole to be better appreciated. Additionally, it enables the development of transgenic therapies that restore normal visual function in diseased retinas by targeting specific cell types (Jepson, et al., 2013).
Fifth, comprehending the diversity and functions of RGC types in the wild-type retina can better inform studies that track how cell types change their function in diseased retinas. One reason for this is because retinal degenerative diseases might affect cell types differentially. For example, a study carried out in a mouse model of glaucoma that found that OFF transient RGCs were more severely impacted than ON transient RGCs (Della Santina, et al., 2013). Understanding the functions of different cell types and how each type is impacted during the onset of a disease will be critical to developing better targeted therapies, especially if the mechanisms that make particular cell types more robust to a disease can be identified and incorporated into current therapies. Additionally, identifying which cell types degenerate more rapidly than other types could also serve as an early screen to identify the onset of the disease. Thus, understanding the functions of cell types in the retina could lead to earlier and improved treatments for retinal degenerative diseases.

1.2.4 Cell type diversity in the rat retina

In this thesis, we study the diversity and function of RGCs in the rat retina. The rat retina offers several advantages for studying the functions of different RGC types. The rat retina contains the major retinal cell classes and many of the same anatomical RGC types as primates and other mammals (Fukuda, 1977; Peichl, 1989; Huxlin & Goodchild, 1997; Sun, et al., 2002a; Sun, et al., 2002b; Wong, et al., 2012). The larger size of the rat eyes compared to mouse allows for easier dissections (Remtulla & Hallett,
The RGCs in the rat are larger than those of mice, which makes spike sorting and cell identification on a MEA more straightforward because spikes with larger amplitudes can be detected and distinguished clearly using clustering methods. The rat is also useful for studying retinal degenerative diseases because several models exist for diseases such as retinitis pigmentosa (RP), glaucoma, and diabetic retinopathy (Morrison, et al., 1997; Yu, et al., 2001; Smith, et al., 2003; Sekirnjak, et al., 2011). Thus, developing an approach to classify RGCs in wild-type rat can provide a baseline for comparison with diseased models. Additionally, it could have potential practical applications such as tracking the changes in functions of specific types as a disease progresses. This can improve the development of targeted therapies like retinal implants, to recreate the functions of damaged cell types.

The Chapters in this thesis are organized in the following manner. Chapter 2 contains a detailed description of the methods used, including data collection, cleaning and analysis. In Chapter 3, we present the classification method developed and the RGC types identified. This classification method addresses the caveats covered in Chapter 1: it identifies cell types reproducibly across preparations, precisely defines RGC types by distinguishing response properties that are robust to variations across experiments, and verifies each cell type by checking for the presence of tiling. In Chapter 4, we quantitatively determine the spatiotemporal RF properties of each RGC type, and provide additional insight into the computations performed by each type. In Chapter 5,
we test how well current models that describe the spiking responses of neurons
generalize to different RGC types, and gain a better appreciation for the computations
performed by each cell type, as well as the potential limitations of these models. Chapter 6 concludes the thesis and addresses future directions.
2. Methods

2.1 Data collection

2.1.1 Tissue preparation and MEA recordings

Adult Long Evans rats were used for the recordings. All experiments followed procedures approved by the Institutional Animal Care and Use Committee of the Salk Institute for Biological Studies. The dissections were performed in darkness under infrared illumination with infrared converters to prevent photopigment bleaching (Anishchenko, et al., 2010; Sekirnjak, et al., 2011). Rats were dark adapted before the experiment and anesthetized with an intraperitoneal injection of Xylazine and ketamine HCl. Animals were then euthanized by decapitation, eyes were enucleated and hemisected posterior to the ora serrata under infrared illumination. The iris, cornea, lens and vitreous were removed, and the eyecup was transferred to oxygenated Ames medium. The orientation of the eyecup was marked using blood vessel landmarks visible through the retina on the choroid, and the retina was isolated from the pigment epithelium. A piece of dorsal retina near the vertical meridian was used to minimize variability across experiments and to target retinal locations with cones expressing mostly M-opsin.

The retina was placed RGC side down on a MEA consisting of 512 electrodes with 60 μm interelectrode spacing, spanning an area of 1.9mm^2 (Figure 2, Litke, et al., 2004). To hold the retina in place, a transparent dialysis membrane was used. The MEA
was then mounted on the recording platform, and continuously perfused with oxygenated Ames’ solution maintained at 30-33°C. The voltage trace recorded on each electrode was bandpass filtered between 80 and 2,000 Hz, sampled at 20 kHz, and stored for off-line analysis. 300-400 RGCs were typically identified in every recording.

2.1.2 Visual stimuli and RGC response properties

Visual stimuli from a CRT video display (Sony Trinitron) refreshing at 120 Hz were focused on the retina via an inverted microscope (Field, et al., 2007). Three different stimuli were used to measure the functional properties of recorded RGCs; each was photopic with a mean intensity of 3000 photoisomerizations/rod/s (Naarendorp, et al., 2010). First, a black and white checkerboard white noise stimulus was used to estimate the spatiotemporal RF by reverse correlation (Figure 3, left; Chichilnisky, 2001). The intensity of each pixel varied independently over space and time, and was drawn from a binary distribution. Each checker of the white noise stimulus was 40x40 μm on the retina and white noise images were updated at 60 Hz. Second, sine wave gratings with a spatial period of 320 μm were drifted in 8 directions at two speeds (150 and 600 μm/s). Each direction was presented 8 times, each time for a duration of 8s, followed by
2s of gray screen (Figure 3, middle). This stimulus identified RGCs that were sensitive to motion and was used to measure the orientation tuning of RGCs (Remtulla & Hallett, 1985; Elstrott, et al., 2008; Zhao, et al., 2013; Baden, et al., 2016). Third, full-field light steps that cycled from white to gray to black to gray every 3 s were used to determine the kinetics of a response to changes in light intensity (Figure 3, right). Fourth, a white noise repeats stimulus that cycled the same sequence of checkerboard white noise stimulus every 10 s was utilized. This stimulus was used to determine the accuracy of a spatiotemporal RF and a linear-nonlinear model at capturing the spiking dynamics of a cell.

Figure 3: Three visual stimuli: left: checkerboard white noise, middle: drifting gratings, right: full field pulses.

2.2 Data cleaning

2.2.1 Spike sorting and neuron identification

Each electrode picked up spikes from many cells, and many adjacent electrodes picked up spikes from the same cell. Spikes were sorted on each electrode to identify as many unique and healthy cells as possible. The spike sorting was carried out on 2 hours of white noise data, and the spike clusters were mapped to the 3 other visual stimuli, as
described below. First, spikes were identified, when the signal amplitude crossed a threshold of 60 micro-volts (Figure 4, left). Second, Principal Components Analysis (PCA) was performed on each electrode, to reduce the dimensionality of the data and find the three most significant variables. Third, spike waveform clusters were identified in this three dimensional space by using an expectation maximization algorithm based on a mixture of Gaussians (Figure 4, middle). Fourth, the resulting clusters were checked and corrected manually in the custom spike sorting software (Field, et al., 2007; Shlens, et al., 2009). Fifth, unique, uncontaminated RGCs were identified by checking for a refractory period (~1.5ms) in the autocorrelation function of each cluster of sorted spikes (Figure 4, right). Sixth, duplicate neurons were identified and removed based on the temporal cross-correlation of the spike trains. RGCs with a firing rate less than 1Hz were also eliminated.

Figure 4: Spike sorting: left: spikes are identified, middle: PCA and clustering, right: autocorrelation functions.

To track the RGCs across different visual stimuli, spike shapes were projected and sorted in the same three-dimensional subspace defined in the white noise stimulus
by PCA. Neuron identity was further confirmed across different stimuli by checking that the electrical image (EI) for each neuron matched across conditions (Petrusca, et al., 2007; Field, et al., 2009). The EI is unique to each cell, and is the average analog waveform generated on each electrode, as the cell spikes. The signal on each electrode is represented by a circle, and the larger the signal, the larger the diameter of the circle (Figure 5). A matched neuron between two stimulus conditions was determined by the EI in stimulus condition “A” with the highest inner product with the neuron in stimulus condition “B” (Field, et al., 2009). A typical experiment resulted in recording and tracking the responses of 300-400 RGCs across three visual stimuli.

![Electrophysiological image](image.png)

**Figure 5: Electrophysiological image.**

### 2.3 Data analysis

#### 2.3.1 Spatiotemporal receptive fields

The spatiotemporal receptive field of each RGC was estimated using reverse correlation analysis to compute the spike triggered average (STA). Frames up to 500ms preceding a spike were included in the analysis. The spatial RF was the set of stimulus pixels whose absolute peak intensity exceeded five robust standard deviations of all pixel intensities (Freeman, et al., 2015). The temporal RF was calculated by averaging these significant stimulus pixels. The temporal RF was then fit using a function that was
a difference of two low pass filters, and normalized by the norm. To fit the spatial RF, the STA frame at the time of maximum deflection of the STA time course from the mean intensity was utilized. A rough estimate of RF center location and radius was generated using a two-dimensional elliptical Gaussian function. Then, an inhibitory surround was introduced, and the fit was re-optimized over the parameters. This fit was used to estimate the RF diameter of the cell. The RF diameter was defined as the diameter of a circle with the same area as the 1SD boundary of a two-dimensional Gaussian fit to the RF center (Chichilnisky & Kalmar, 2002). It was computed by taking the geometric mean of the standard deviations of the fit along the major and minor axes.

2.3.1.1 Spatial RF mosaics

To plot the RF mosaics, RF contours were used instead of the two-dimensional Gaussian fit. These took into account the shape of the RF, as some cells had non-Gaussian shaped RFs. RFs were first filtered by convolving with a two-dimensional Gaussian function, filter radius 1 stimulus pixel. After spatial smoothing, contour lines were linearly interpolated in each RF using a fixed contour level for all cells (Gauthier, et al., 2009a).

2.3.1.2 Spatial RF profiles

RF profiles (Figure 28 e) of each cell type were computed by averaging together the normalized distances between all nearest neighbors (Gauthier, et al., 2009b). First, the center point of each spatial RF was computed by taking the center of mass of all
significant stimulus pixels that had a pixel intensity at least 5 standard deviations above the mean. Second, the amplitude of the STA was normalized to have unit variance. Third, the distance between each cell in a mosaic, and its nearest neighbor was computed. This distance was normalized, so that the nearest neighbor was exactly 1 unit away. Fourth, the amplitudes of the pixels in the RF of each cell in the mosaic and its nearest neighbor were extracted. The RF pixel intensity was a continuous function, with a pixel intensity at each point along the line connecting the two cells. Finally, the RF profiles were averaged for all reference cells within each type. The amplitude was normalized to have unit variance.

2.3.1.3 Parameterization of the temporal RF

The time-to-peak (Figure 6 a) and time-to-trough (Figure 6 b) were taken from the global maximum and minimum, respectively, in the temporal RF. The zero crossing was calculated as the time closest to the spike at which the temporal RF transitioned from positive (negative) to negative (positive) values (Figure 6 c). The maximum and minimum values were taken as the global maximum and minimum in the temporal RF, respectively (Figure 6 d, e). The gradient was defined as: (maximum value - minimum value) / (time-to-peak - time-to-trough). A phasic index (PI) was calculated from the temporal RF as the sum of the positive and negative areas divided by the sum of their absolute values (Fig 6 f, g: \((f+g) / (|f| + |g|)\)). The PI = 1 or -1 for a temporal RF that consists of all positive or negative values, respectively. For a temporal RF that has equal
area above and below zero, the PI = 0. The normalized area was the absolute area under the temporal RF (Figure 6 f, g), normalized by the norm.

![Temporal RF parameters.](image)

**2.3.1.4 Parameterization of the spatial RF**

Spatial RF area was calculated from the stimulus pixels (stixels) that exceed 5 standard deviations above or below the mean of the STA. A convex hull was drawn around the edges of this region and the area of this hull was used to estimate the area of the spatial RF. The spatial regularity index (SRI) for the RF shape was calculated as:

\[
\text{SRI} = \frac{\text{area(significant stixels)}}{\text{area (convex hull)}},
\]

which was useful for identifying non-Gaussian RFs. Gaussian RFs will exhibit high SRIs, while non-convex RFs will exhibit low SRIs. Finally, a spatial contrast index (SCI) was defined as:

\[
\text{SCI} = 1 - \frac{1}{|((\text{number of positive stixels}) - (\text{number of negative stixels})) / \text{total number of stixels}|}
\]

and was used to identify RFs that had on and off sub regions in the spatial RF.

**2.3.2 Static nonlinearities**

Static nonlinearities (SNLs) were computed by convolving the spatiotemporal RFs with the checkerboard noise stimulus. This yielded an instantaneous generator
signal for each frame that was used to generate a histogram of observed spike counts for each generator signal. This histogram was fit with a logistic function. The slope (b) and offset (a) were parameters from the logistic function fit to the SNL: \( c/ (1+\exp(-b(x-a))) \).

To check that the static nonlinearity was accurately fit, simulated spikes were generated from a model Linear-Nonlinear Poisson neuron in response to a checkerboard white noise stimulus. A logistic function was used in the simulation for the SNL. When total spike counts were matched between simulated and real neurons, the model fitting produced estimates of the slope and offset within 1% of the values set in simulation. Moreover, as an additional control, it was also shown that the results were mostly consistent when the SNLs were fit using a different function: an exponential of the form \( \exp(ax+b) \).

### 2.3.3 White noise repeats responses

Spike rasters were plotted for each cell’s response to the white noise repeats stimulus. The PSTH was computed by taking the average of the cell’s firing rate over 60 repeats of the stimulus, with a bin size of 16.6ms.

### 2.3.4 Linear-nonlinear model

We used a linear-nonlinear model to predict the firing rate of the cell. This is a widely used model for visual response, and consists of a single linear filter, followed by an instantaneous nonlinear function (Chichilnisky, 2001; Pillow, et al., 2005). The linear filter was the spatiotemporal RF of the cell, estimated using STA. The white noise
repeats movie was filtered through the spatiotemporal RF, to get a generator signal for each frame. The generator signal was then passed through the SNL to get a frame by frame predicted firing rate for the cell. The SNL accounted for response nonlinearities such as rectification and saturation. The linear and nonlinear functions were computed from the response of the cell to the white noise stimuli, and were tested on new stimuli (the white noise repeats).

2.3.4.1 Quantifying model performance

To measure how well the predicted firing rate mirrored the actual firing rate of the cell, we computed these parameters:

a) R squared = 1 - Residual Sum of Squares / Total Sum of Squares;

Residual Sum of Squares = Sum [(Predicted FR - Actual FR) ^2];

Total Sum of Squares = Sum [(Actual FR - Mean of Actual FR) ^2]; s

b) Pearson’s Correlation Coefficient = Covariance (Predicted FR, Actual FR) / [Std (Predicted FR)* Std (Actual FR)]

2.3.5 Space-time plots and center-surround kinetics

The space-time plots collapse the 3-dimensional spatiotemporal RFs into 2 dimensions to visualize the change in the spatial RF over 350ms before a spike. First, the entire spatiotemporal RF is filtered and a 21x21 (924 microns x 924 microns) stimulus pixel region around the center of mass of the cell’s RF is cropped. Second, the average 3-dimensional spatiotemporal RF of each RGC type is computed by averaging together all
the cropped and filtered spatiotemporal RFs of all cells of that type across all recordings. Third, the 3-dimensional spatiotemporal RF is collapsed to 2 dimensions by averaging together the intensities of all pixels along the x axis (averaging the intensities of all pixels along the y axis produced almost identical space-time plots). The center and surround kinetics were computed by taking two cuts through the space time plot: the planes with the maximum (center) and minimum (surround) average pixel intensities.

### 2.3.6 Space-time separability and singular value decomposition

The space-time separability of RFs estimated by the STA was determined using singular value decomposition (SVD; Stewart, 1993; Depireux, et al., 2001; Emerson & Vaughn, 2011). SVD factorizes a matrix into a rank-ordered set of vector pairs whose outer products are weighted and linearly combined to reproduce the original matrix. A perfectly space-time separable RF will produce a single pair of non-zero vectors capturing the spatial and temporal RFs. In general, noise in the STA will cause the estimated RF to be inseparable. To determine whether deviations from a perfectly separable RF were consistent with measurement noise alone, a distribution of random spatiotemporal filters was generated by bootstrapping (Schwartz, et al., 2006). First, the spike train was randomly shifted to compute a new spatiotemporal RF that consisted of only noise. Second, SVD was performed on this RF and the singular values were extracted. This was repeated 200 times, and the 95% confidence intervals on the largest and smallest singular values were estimated. Any singular values obtained from the true
spike train that did not lie within this interval were deemed as significant and not due to measurement noise alone. All RGC types exhibited deviations from separability that could not be accounted for by measurement noise alone.

2.3.7 RF expansion

Some RGC types exhibited a spatial STA that expanded with time. To quantify the magnitude of this effect across RGC types, RF area was measured for every RGC type over 5 frames preceding the peak frame of the temporal RF. First, the STA of each cell was filtered, with filter radius 0.75 stimulus pixels. Then, a 21x21 (924 microns x 924 microns) stimulus pixel region around the center of mass of the cell’s RF was cropped, across the 30 STA frames. The STAs of all cells of a type were averaged together, to get an average STA for each cell type. Each frame of the STA had an associated contour, which captured the spatial extent of the RF. The area inside that contour was computed for each RGC type over 3 frames preceding and 1 frame following the peak frame of the temporal RF. The fraction of spatial RF expansion relative to the earliest frame was quantified on a per frame basis, over 3 frames. The slope of the best fit line over these frames quantified the expansion rate for each cell and the mean across cells was compared over cell types. The average RF area was confirmed as an accurate estimate of the RF area of cells of a type by bootstrapping.
2.3.8 Interspike intervals

For every cell, a distribution of the interspike interval (ISI) was generated from the white noise stimulus data. The ISI is the probability that a spike was fired within a certain time interval \([t + dt]\), given that a spike occurred at time \(t\). The smallest \(dt\) was 1 ms, and the ISI was computed for up to 100 ms after a spike was fired.

2.3.8.1 Parameterization of the interspike intervals

PCA was performed on the ensemble of ISI distributions. The projection of each ISI distribution along the first principal component was used as a parameter for classifying RGCs.

2.3.9 Drifting grating responses

Spike rasters were plotted for each cell’s response to each direction of the drifting grating stimulus. There were 8 trials in every direction. The peri-stimulus time histogram (PSTH) was computed for each direction of the drifting grating stimulus, by taking the average of the cell’s firing rate over all 8 repeats of the stimulus, with a bin size of 100ms. The firing rates in response to drifting gratings were calculated by dividing the total spike count by the number of stimulus repeats (8), directions (8) and length of time that the grating was presented to the retina (8 or 10 s).

2.3.9.1 Magnitude of vector sum and polar plots

Direction selective ganglion cells in the rat were identified by calculating the magnitude of their vector sum to 2 speeds of the drifting grating stimulus. To do this,
we first summed the total number of spikes fired in each direction (Figure 7). Then, the spikes numbers were normalized by the direction with the maximum number of spikes, so each direction had a value between 0 and 1. A vector was generated for each direction of the drifting grating, where the magnitude of each vector was the normalized total number of spikes. Each direction had a vector with a magnitude, and this was represented by the polar plot (Figure 7, middle). The vector sum was evaluated by summing all 8 vectors, which gave 1 vector with a magnitude and direction for each cell for each spatial and temporal period of the drifting grating stimulus. The magnitude of the vector sum was high for DS-RGCs and close to zero for non-DS-RGCs. To identify DS-RGCs in a quantitative manner, the log of the magnitude of the vector sum was computed for each cell to both speeds of the drifting grating stimulus, and a mixture of two Gaussians was fit to these points (described in more detail below).

**Figure 7:** Drifting grating responses to 8 directions for an example RGC, middle: polar plot (blue) and vector sum (red).

### 2.3.9.2 Orientation selective index

The orientation selective index (OSI) was calculated as the ratio of: 

\[ \frac{(R_{\text{pref}} - R_{\text{orth}})}{(R_{\text{pref}} + R_{\text{orth}})} \]

where \( R_{\text{pref}} \) was the mean firing rate of the 2 directions
corresponding to the preferred orientation, and Rorth was the mean firing rate of the two directions orthogonal to the preferred orientation (Zhao, et al., 2013). The OSI was then scaled by the average trial-to-trial variability across all directions of the drifting gratings. This was computed by taking the dot product of the PSTH across all trials of each direction of the drifting grating. If cells fired consistently across trials, the dot product would be close to one, if not, it would be close to zero. Eight dot products were computed (one for each direction) for each cell, which were then averaged together and multiplied by the OSI. This scaled OSI was then averaged across all cells within a type in each recording. A two sample t-test was used to determine if the OSIs of OS RGC types were statistically different from other RGC types (Dodge, 2008; Gauthier, et al., 2009a).

2.3.9.3 Direction tuning curves

To determine how well the spatial RF predicted orientation tuning of the OS RGC types, the dot product of the spatial RF of each cell with the gratings at 8 different orientations was computed. This dot product-orientation tuning curve was averaged across all cells within a type, normalized by the norm, and compared to the actual tuning curves from the firing rate. The actual tuning curve for each cell was computed by averaging together the total number of spikes fired at each direction of the drifting grating stimulus. The tuning curves of all cells within a type were then averaged together, and normalized by the norm.
2.3.10 Full field pulse responses

Spike rasters were plotted for each cell’s response to the full field pulses, and there were up to 50 repeats. The peri-stimulus time histogram (PSTH) was computed by taking the average of the cell’s firing rate over all 50 repeats of the stimulus, with a bin size of 100ms.

2.4 RGC classification approach

The classification approach consisted of two steps: a feature selection process followed by a serial, quantitative classification. The feature selection process identified response properties that robustly isolated one or a small number of RGC types from all other types (e.g. isolating DS-RGCs from non-DS-RGCs). The quantitative classification clustered neurons using these features and a two-Gaussian mixture model.

2.4.1 Feature selection (step one)

The feature selection process was performed as follows: First, to identify relevant features, high-dimensional data (from 300 - 400 RGCs in each recording) was compressed and visualized in a lower dimensional space by PCA. PCA was performed on the temporal RFs and ISIs of each cell. The three dimensions of highest variance were plotted, and cells that formed clusters in this space were identified. Inspection of these cell clusters revealed groups of RGCs with distinct functional properties. Each cell cluster was verified to be a putative cell type by checking for the presence of regular spatial RF mosaics.
Once putative RGC types were identified, the responses of all RGCs to the 3 visual stimuli were inspected. To distinguish each RGC group from other RGCs, a maximum of three relevant and meaningful features were computed from the spatial and temporal RFs, drifting grating responses and ISIs. These features were chosen via visual inspection, parametrization, and based on properties known to distinguish RGC types (e.g. direction tuning and shape of the temporal RF; Devries & Baylor, 1997; Carcieri, et al., 2003; Farrow & Masland, 2011). Three features were selected for the following reasons: a) Using one to two features to classify cell types did not allow precise boundaries to be defined that clearly distinguished each type. b) Selecting more than three features did not change or sometimes worsened the performance of the clustering algorithm, because the amount of data needed increased exponentially with the number of features (see step two). c) The clusters could be visually inspected by plotting them in a three-dimensional space.

Once a set of response features were identified that clearly separated one group of RGCs from the others, the spatial RFs (or EIs) of the grouped RGC were inspected to check whether they were regularly spaced. If grouped RGCs were regularly spaced, the features used were saved for quantitative clustering (see step two). Performing feature selection before quantitative classification improved the performance of the clustering algorithm because it resulted in well-separated clusters that precisely identified each RGC type. This enabled the classification approach to generalize well to new data sets,
even when the shapes of clusters changed depending on the state of the preparation, minimized misclassification rates, and allowed the results to be replicated across multiple recordings.

### 2.4.2 Quantitative clustering (step two)

To quantitatively cluster (classify) each RGC type, a two Gaussian mixture model (GMM) was fit in the same two or three dimensional feature space defined above. The GMM allowed boundaries to be drawn between clusters according to the maximum likelihood that RGCs belonged to one Gaussian distribution or the other. The putative RGC groups identified in the feature selection step were the initial condition for the mixture model fit. RGC types were classified one at a time in a serial fashion, similar to a previous study (Carcieri, et al., 2003). Once a type was classified, the next type would be identified from the remaining cells and so on, until no more types could be identified. Classification was terminated when a significant cluster and its corresponding RF mosaic could not be identified from the remaining data. Classifying cell types serially prevented overfitting of the data, and avoided ambiguity in choosing the number of clusters. Each cluster was tested for statistical significance, and the irreducibility of each type was verified by checking for the presence of regular spatial RF tiling (see below). The cells’ responses to the three visual stimuli were also examined, and it was found that all cells of a type responded in a very similar manner to the three visual stimuli (Data shown in Chapter 3). The classification was cross-validated by using the same
features to cluster RGC types in 2 additional recordings from other animals (total of 3 recordings). Additionally, the classification approach was tested on 3 rat recordings from a different lab to ensure that it generalized well to new datasets. The order of this serial classification and the response parameters that consistently identified RGCs across recordings is discussed in Chapter 3.

2.4.3 Verifying RGC types

Clustered RGCs were identified as a “type” if their spatial RFs uniformly sampled visual space. This was quantified by plotting their normalized nearest neighbor distribution (NNND; Devries & Baylor, 1997; Field, et al., 2007; Gauthier, et al., 2009b). The NNND is defined as $2R / (S_1 + S_2)$ where R is the distance between the centroid of the Gaussian fit to the receptive field of the two nearest neighbors in the mosaic, and $S_1$ and $S_2$ are SDs of the fits measured along the line connecting the centroids. The regularity of RF sampling was demonstrated by ensuring that there was an exclusion zone in the NNNDs, signifying minimal overlap between neighbors.

2.4.3.1 Electrophysiological image mosaics

For DS-RGCs, the spatial RFs were unreliable estimated by the STA. This is because most DS-RGCs are ON-OFF, and therefore the STA signal was weak. For these cells, regular spacing was tested by inspecting their electrophysiological images (EIs). The soma location was estimated from the EI by computing the center of mass of the electrical signal between the electrode with the largest signal and its 6 nearest neighbor
electrodes. This prevented the estimated cell locations from being registered to the
electrode locations. The nearest neighbor distribution (NND) was then computed on
these estimated cell locations.
3. Functional diversity and classification of rat retinal ganglion cells

3.1 Introduction

Retinal ganglion cells send information about the visual scene to the brain. Morphological studies have shown that there are at least 20 retinal ganglion cell (RGC) types in the rodent retina, while functional and genetic studies have shown that there are between 5 - 30 distinct RGC types (Chapter 1; Devries & Baylor, 1997; Huxlin & Goodchild, 1997; Rockhill, et al., 2002; Sun, et al., 2002; Carcieri, et al., 2003; Badea & Nathans, 2004; Dacey, 2004; Coombs, et al., 2006; Volgyi, et al., 2009; Farrow & Masland, 2011; Baden, et al., 2016). Morphological, functional and genetic studies have failed to yield a consistent picture of cell type diversity and function in the retina, because the field still lacks a standardized approach to classify RGC types. A standardized classification approach, that defines clear and precise boundaries between cell types is needed, and should be based on traits that are quantifiable and precise, reproducible across experiments and can be validated (Wichterle, et al., 2013). An approach like this can provide an unambiguous appreciation of cell type diversity, and a better understanding of the organization and function of the retina. It also has some potential practical benefits such as tracking the same types across different labs, disease states or genetic manipulations states. Moreover, a rigorous and systematic classification approach might reveal unexpected cell types, and allow a more accurate identification of previously under-appreciated RGC types.
In this section, we utilize large-scale parallel recordings to record from hundreds of RGCs, and present a novel quantitative approach to classify RGC types. This approach balances quantitative classification with careful feature selection, verifies cell types by confirming the presence of receptive field mosaics, and was cross-validated across several preparations, with a 95% accuracy rate. These features are not cumulatively present in previous functional classifications of RGCs in any species (Devries & Baylor, 1997; Carcieri, et al., 2003; Farrow & Masland, 2011; Baden, et al., 2016). Using this approach, we established that there were at least 13 clearly defined cell types in the rat. The approach also led to the discovery of 3 novel types: an OFF vertical orientation selective (OS) type, an OFF horizontal OS type, and an OFF cell type that prefers expansion. Developing an approach to classify RGC types and identifying new cell types is fundamental to further our understanding of how the retina works and the role it plays in visual processing.

3.2 Results

We developed a serial classification approach that was reproducible across recordings, quantitatively distinguished RGC types, and was verified by checking that the spatial RFs of each type tiled space. The classification approach is described in detail in Chapter 2.

The approach yielded 13 RGC types across 6 recordings. Data from 3 recordings are shown below. The RGC types consisted of four DS cell types, 3 ON types, 3 OFF
types, an OFF vertical orientation selective (OS) type, an OFF horizontal OS type, and an OFF cell type that preferred expansion. In the sections below, we present the order of the serial classification, highlight the features that distinguished each type, and discuss the unique functional properties of each type.

3.2.1 Classification of direction selective RGCs

First, the DS-RGCs were identified based on their responses to drifting gratings. DS-RGCs respond preferentially to motion in one direction, while non-DS-RGCs respond equally to motion over all directions. To differentiate DS-RGCs from non-DS-RGCs, the magnitude of the vector sum of the cells over 8 directions of the drifting grating stimulus, at each of two temporal periods was computed (see Methods, Chapter 2). Plotting these vector magnitudes across all recorded RGCs revealed two clusters (Figure 8a). DS-RGCs had a vector sum with a large magnitude and comprised the cluster on the top-right, while non-DS-RGCs made up the cluster of cells on the bottom-left. The boundary between clusters was determined by fitting the data with a 2-Gaussian mixture model (Figure 8 a red vs. blue). The cluster of cells in red was the DS-RGCs: they had high vector magnitudes (red) and exhibited preferred directions along the four cardinal axes (Figure 8 a, inset), consistent with the four types of DS-RGCs found in previous results (Vaney, et al., 2001; Elstrott, et al., 2008). The cluster of cells in blue was the non-DS-RGCs: they had low vector magnitudes and random preferred directions.
Morphological and physiological studies have indicated that DS-RGCs with the same direction preference regularly sample space in a mosaic-like arrangement and constitute a cell type (Vaney, 1994; Fiscella, et al., 2012; Vaney, et al., 2012). To verify that there were four types of DS-RGCs, the EIs of the cell locations were plotted for each cell (Figure 8 b, retina 1). Supporting this conclusion, the electrical images (EIs) of the cell locations were spaced regularly when separated by direction (Figure 9 a), while the spacing was irregular when all directions were combined (Figure 9 b).

Figure 8: a: Classification of DS-RGCs from one recording: Red points (38) are DS-RGCs, blue points (286) are other RGCs. Red arrows indicate direction preference of DS-RGCs. b: EI mosaics of DS-RGCs for each preferred direction from 3 different recordings.

Figure 9: a: NNDs of DS-RGCs for each direction (data combined across all 3 recordings). b: NNDs of all DS cells within one recording.
To ensure that this approach could reproducibly identify DS-RGCs, these results were replicated in two additional recordings from different retinas. The magnitude of vector sum distinguished the DS-RGCs from other RGCs in all the recordings (Figure 8b, retina 2 and retina 3). As nearly all DS-RGCs (93 of 107) had ON-OFF responses to full-field light steps, we did not distinguish between ON and ON-OFF DS-RGCs. We assume the DS-RGCs are predominantly ON-OFF due to the low encounter rate of ON DS-RGCs (Sun, et al., 2006). These results suggest that the extracellular recordings and spike sorting methods used here may be biased against sampling from ON DS-RGCs. Nevertheless, this set of features could quantitatively, verifiably and reproducibly identify DS-RGCs from non-DS-RGCs across all recordings.

### 3.2.2 Classification of ON and OFF RGCs

After classifying the DS-RGCs, the remaining non-DS-RGCs were segregated into ON and OFF cells. ON cells prefer an increment in light intensity; while OFF cells prefer a decrement in light intensity before they fire a spike. The parameters used to segregate these cells were the time to trough (OFF cells had a time-to-trough closer to the spike time than ON cells; Figure 10 b, c) and the magnitude of the largest value in their temporal RF (ON cells had a peak value with a positive magnitude, OFF cells had a peak value with a negative magnitude; Figure 10 b, c). A two-Gaussian mixture model was fit, and segregated the ON (in red) and OFF cells (in blue; Figure 10 a). The extensive overlap and irregularity in the RF mosaics showed that ON and OFF cells did
not form a cell type, and could be further sub classified (Figure 10 d, e). The ON cell types were classified in serial fashion, followed by the OFF types, and the order of the classification is presented below.

![Figure 10](image)

Figure 10: a: Classification of ON and OFF RGCs. b: Temporal RFs of OFF RGCs. c: Temporal RFs of ON RGCs. d: RF mosaics of OFF RGCs (n = 160). e: RF mosaics of ON RGCs (n = 123).

### 3.2.3 Classification of ON RGCs

Serial classification of ON RGCs revealed 3 types that differed systematically in their response properties. Below we describe the classification of these cells and compare their functional properties.

#### 3.2.3.1 ON brisk sustained RGCs

Inspection of the responses of ON RGCs to the three visual stimuli revealed cells with high mean firing rates to drifting gratings (Figure 11 a1,a2) and weakly biphasic temporal RFs (Figure 11 b1, b2). To differentiate these cells from other ON RGCs, three response parameters were extracted: (1) the phasic index (PI), which quantified the
shape of the temporal RF, (2) the mean firing rate to a slowly drifting grating (150 microns/s), and (3) the mean firing rate to a rapidly drifting grating (600 microns/s, see Methods). A two-Gaussian mixture model was fit to the data, and quantitatively distinguished this cell type from other ON RGCs (Figure 11 e1, e2, red circles). To conform with previous naming conventions from the rabbit and other mammals (Devries & Baylor, 1997; Rockhill, et al., 2002), we refer to these cells as ON brisk sustained (BS) RGCs because of their short latency (brisk) temporal RFs (Figure 11 b1, b2) and temporally sustained responses to drifting gratings and steps in light intensity (Figure 11 a1, a2, Figure 12 e1, e2).

Figure 11: a1-e1: ON BS RGCs from one recording. a1: Drifting Grating Response (10 Hz, 2s). b1: Temporal RF (0.3 a.u, 125 ms). c1: ISI (0.1 a.u. 10ms). d1: Spatial RF. e1: Red: ON BS RGCs (36), blue: other ON RGCs (87). a2-e2: ON BS RGCs (22) from second recording.

An analysis of the spatial RF locations of these RGCs revealed they formed a mosaic in each recording (Figure 12 a1, a2; Devries & Baylor, 1997; Field, et al., 2007;
Anishchenko, et al., 2010). Regular spacing was further confirmed by the presence of an exclusion zone followed by a peak in their normalized nearest neighbor distribution (Figure 12 b1, b2). Importantly, no information about the spatial location of these cells was used to classify them, thus the observation of a mosaic was not dictated by the analysis. This organization indicates that these RGCs correspond to a morphologically distinct type and that these cells cannot be further sub classified; they are irreducible (Wassle, et al., 1981; Devries & Baylor, 1997; Sanes & Masland, 2015).

**Population Properties of ON Brisk Sustained RGCs**

Figure 12: a1-e1: ON BS RGCs from one recording. a1: RF mosaics. b1: NNNDs. c1: Average Temporal RFs (0.3 a.u. 125ms), Shaded region is +/- 1 s.d. d1: Mean ISI. e1: Average response to full field light steps (0.1 a.u., 2s). a2-e2: ON BS RGCs from second recording.

ON brisk sustained RGCs had branchy, non-Gaussian shaped spatial RFs with large RF diameters (Figure 11 d1, d2). Their temporal RFs were weakly biphasic and highly stereotyped, deviating minimally from the mean shape (Figure 12 c1, c2). Other
properties not used to classify these cells were also highly stereotyped. For example, the ISI distribution (Figure 12 d1, d2) and the sustained responses to full-field light steps (Figure 12 e1, e2) varied little across cells and across recordings.

3.2.3.2 ON brisk transient RGCs

Examining the remaining ON RGCs revealed many cells with lower mean spike rates to drifting gratings (Figure 13 a1, a2) and more biphasic temporal RFs (Figure 13 b1, b2) compared to the ON brisk sustained cells. Plotting two parameters of their temporal RFs (time to zero crossing, and minimum value) against the shape of the inter-spike interval (ISI) distribution, reliably classified this cell type with a two-Gaussian mixture model in each recording (Figure 13 e1, e2). We refer to these cells as ON brisk transient (BT) cells.

Figure 13: a1-e1: ON BT RGCs from one recording. a1: Drifting Grating Response (10 Hz, 2s). b1: Temporal RF (0.3 a.u, 125 ms). c1: ISI (0.2 a.u. 10ms). d1: Spatial RF. e1: Red: ON BT RGCs (37), blue: other ON RGCs. a2-e2: ON BT RGCs (35) from second recording.
Like the ON brisk sustained cells, the spatial RFs of ON brisk transient cells exhibited a mosaic organization (Figure 14 a1, a2) and regular spacing (Figure 14 b1, b2), indicating that they were also an irreducible type (Wassle, et al., 1981; Anishchenko, et al., 2010). To demonstrate the regularity of RF tiling and to fill the gaps in the RF mosaic, the spatial RFs of cells that were identified in the white noise stimulus, but could not be tracked across the other stimuli are shown in light gray. The dark gray spatial RFs correspond to cells that were tracked across all three visual stimuli (Figure 14 a1, a2).

ON brisk transient RGCs displayed sharply peaked ISI distributions (Figure 14 d1, d2), and transient responses to full-field steps (Figure 14 e1, e2) and drifting gratings (Figure 14 a1, a2). They also had more Gaussian-shaped spatial RFs, (Figure 13 d1, d2) and more biphasic temporal RFs than the ON brisk sustained RGCs (Figure 14 c1, c2).

**Population Properties of ON Brisk Transient RGCs**

Figure 14: a1-e1: ON BT RGCs from one recording. a1: RF mosaics. b1: NNNDs. c1: Average Temporal RFs, Shaded region is +/- 1 s.d. d1: Mean ISI (0.2 a.u., 10ms). e1: Average response to full field light steps (0.2 a.u., 2s). a2-e2: ON BT RGCs from second recording.
3.2.3.3 ON sluggish transient RGCs

Investigating the remaining unclassified ON RGCs revealed a third set of cells with more sluggish and biphasic temporal RFs (Figure 15 b1, b2). Two parameters of their temporal RFs (time to zero crossing and minimum value) and the shape of the ISI distribution (Figure 15 c1, c2) revealed these cells were distinct from other ON cells (Figure 15 e1, e2).

![Example ON Sluggish Transient RGCs and Classification](image)

**Figure 15:** a1-e1: ON ST RGCs from one recording. a1: Drifting Grating Response (10 Hz, 2s). b1: Temporal RF (0.3 a.u, 125 ms). c1: ISI (0.1 a.u. 10ms). d1: Spatial RF. e1: Red: ON ST RGCs (12), blue: other ON RGCs. a2-e2: ON ST RGCs (11) from second recording.

These RGCs were sampled with lower efficiency in these recordings, however their spatial RFs were consistent with a mosaic organization (Figure 16 a1,a2) and regular spacing (Figure 16 b1, b2) These cells exhibited highly stereotyped interspike interval distributions (Figure 16 d1, d2), small spatial RFs (Figure 15 d1, d2), strongly biphasic temporal RFs (Figure 16 c1, c2), and very transient responses to full-field light.
steps (Figure 16 e1, e2) and drifting gratings (Figure 15 a1, a2). We refer to these cells as ON sluggish transient (ST) cells because their temporal RFs had slower kinetics than ON brisk transient cells.

**Figure 16**: a1-e1: ON ST RGCs from one recording. a1: RF mosaics. b1: NNNDs. c1: Average Temporal RFs, Shaded region is +/- 1 s.d. d1: Mean ISI (0.2 a.u., 10ms). e1: Average response to full field light steps (0.2 a.u., 2s). a2-e2: ON ST RGCs from second recording.

These three RGC types accounted for 67 +/- 3% of all ON cells across recordings. The remaining ON RGCs could not be reliably classified because the number of cells with similar response properties were small, which precluded a quantitative classification and checking for a mosaic organization.

### 3.2.4 Classification of OFF RGCs

In this section we describe the classification of OFF RGC types and their functional organization.
3.2.4.1 OFF brisk sustained RGCs

Inspection of individual OFF RGCs revealed cells with weakly biphasic temporal RFs (Figure 17 b1, b2). These cells also had strongly modulated firing rates in response to drifting gratings, similar to ON brisk sustained cells (Figure 17 a1, a2). Thus, we used the same response parameters (phasic index, drifting grating responses) to classify these cells (Figure 17 e1, e2). The cells could be classified reproducibly in all 3 recordings (2 shown), and their spatial RFs exhibited a mosaic organization in each recording (Figure 18 a1, a2), indicating an irreducible type. We refer to them as OFF brisk sustained (BS) RGCs.

Example OFF Brisk Sustained RGCs

Classification

Figure 17: OFF BS RGCs from one recording. a1: Drifting Grating Response (10 Hz, 2s). b1: Temporal RF (0.3 a.u, 125 ms). c1: ISI (0.1 a.u. 10ms). d1: Spatial RF. e1: Red: OFF BS RGCs (34), blue: other OFF RGCs (126). a2-e2: OFF BS RGCs (23) from second recording.

Off Brisk Sustained RGCs had large RF diameters, slightly non-Gaussian spatial RF shapes (Figure 17 d1, d2), sustained responses to full field light decrements (Figure
18 e1, e2) and drifting gratings (Figure 17 a1, a2), and weakly biphasic temporal RFs (Figure 18 c1, c2). These response properties were highly stereotyped across cells and across recordings (Figure 18 c1, c2, e1, e2). However, the ISIs were more variable within and across recordings, which may indicate that these cells have more variable spiking dynamics or that their dynamics are more susceptible to the ex vivo conditions of these recordings (Figure 18 d1, d2).

3.2.4.2 OFF brisk transient RGCs

Among the remaining OFF RGCs, there was a group of cells that was less strongly modulated by drifting gratings (Figure 19 a1, a2) had strongly biphasic temporal RFs (Figure 19 b1, b2), transient responses to decrements of the full field light
steps (Figure 20 e1, e2), and large Gaussian-shaped spatial RFs (Figure 19 d1, d2). These were very similar to the ON brisk transient RGCs.

Figure 19: a1-e1: OFF BT RGCs from one recording. a1: Drifting Grating Response (10 Hz, 2s). b1: Temporal RF (0.3 a.u, 125 ms). c1: ISI (0.1 a.u. 10ms). d1: Spatial RF. e1: Red: OFF BT RGCs (52), blue: other OFF RGCs. a2-e2: OFF BT RGCs (41) from second recording.

However, they were not classified using the same parameters that were used to classify the ON brisk transient RGCs because cell-to-cell variability in their ISIs precluded using this when classifying these cells. Instead, parameters of the temporal RF (time-to-peak, time-to-trough, and phasic index) were extracted, and revealed that these cells were a distinct type in each recording (Figure 19 e1, e2). The spatial arrangement of these RGCs also exhibited a mosaic organization (Figure 20 a1, a2). Their transient responses to full-field light steps (Figure 20 e1, e2) and their similarity to ON brisk transient RGCs, led to naming these OFF brisk transient (BT) RGCs.
Figure 20: a1-e1: OFF BT RGCs from one recording. a1: RF mosaics. b1: NNNDs. C1: Average Temporal RFs. d1: Mean ISI (0.2 a.u., 10ms). e1: Average response to full field light steps (0.2 a.u., 2s). a2-e2: OFF BT RGCs from second recording.

3.2.4.3 OFF sluggish transient RGCs

Another group of RGCs exhibited strongly biphasic temporal RFs (Figure 21 b1, b2), but with slower kinetics compared to the OFF brisk transient RGCs and their spike rates were minimally modulated by drifting gratings (Figure 21 a1, a2). They were identified with a two-Gaussian mixture model fit to three parameters of their temporal RF: time to peak, phasic index, and gradient between the peak and trough (Figure 21 e1, e2).

These cells were not sampled with high efficiency in these recordings, but their spatial organization was consistent with a mosaic (Figure 22 a1, a2). We refer to these as OFF sluggish transient (ST) RGCs, due to their similarity to the ON sluggish transient
RGCs. OFF sluggish transient RGCs had Gaussian-shaped spatial RFs with small RF diameters (Figure 21 d1, d2), very transient responses to decrements in the full field light steps (Figure 22 e1, e2), and could be identified across all 3 preparations.

**Example OFF Sluggish Transient RGCs**

![Diagram of example OFF sluggish transient RGCs](image)

**Classification**

![Classification diagram](image)

**Figure 21**: a1-e1: OFF ST RGCs from one recording. a1: Drifting Grating Response (10 Hz, 2s). b1: Temporal RF (0.3 a.u., 125 ms). c1: ISI (0.1 a.u. 10ms). d1: Spatial RF. e1: Red: OFF ST RGCs (12), blue: other OFF RGCs. a2-e2: OFF ST RGCs (7) from second recording.

**Population Properties of OFF Sluggish Transient RGCs**

![Population properties diagram](image)

**Figure 22**: a1-e1: OFF ST RGCs from recording 1. a1: RF mosaics. b1: NNNDs. c1: Average Temporal RFs. d1: Mean ISI (0.2 a.u., 10ms). e1: Average response to full field light steps (0.2 a.u., 2s). a2-e2: OFF ST RGCs from recording 2.
3.2.4.4 OFF expanding RGCs

Three more RGC types were identified among the remaining OFF cells. The first of these exhibited a biphasic temporal RF (Figure 23 a1, a2) and a sharply peaked ISI (Figure 23 b1, b2). These cells could be distinguished from other OFF cells based on the first principal component of the ISI distribution, and two features of their temporal RF: the time-to-trough and the minimum value (Figure 23 d1, d2). These cells tiled space, indicating they were an irreducible type (Figure 23 e1, e2), and were identified in all 3 recordings. Interestingly, these RGCs had spatial RFs that expanded in time (analyzed in Chapter 4). Additionally, they were suppressed by full-field increments and decrements in light intensity to white and black (Figure 23 c1, c2). They were also suppressed by lateral motion - they hardly fired to all 8 directions of the drifting grating stimulus, and could potentially correspond to the equivalent of the looming RGCs found in mouse.

![Off Expanding RGCs: Population Properties](image)

Figure 23: a1-e1: OFF Exp RGCs from recording 1. a1: Temporal RFs. b1: Mean ISI (0.2 a.u., 10ms). c1: Average response to light steps (0.2 a.u., 2s). d1: Red: OFF Exp RGCs (23), blue: other OFF RGCs (51). e1: RF mosaics. a2-e2: OFF Exp RGCs (11) from recording 2.
3.2.4.5 OFF orientation selective RGCs

Classification of the remaining OFF cells produced 2 additional types with responses that were tuned to horizontal and vertical orientations, respectively (OS-RGCs). We demonstrate that each type tiles space, and exhibits distinct functional properties.

3.2.4.6 OFF horizontal orientation selective RGCs

The first orientation selective (OS) type was classified from the remaining RGCs based on three parameters of the temporal RF: time-to-trough, time-to-peak, and phasic index (Figure 24 d1, d2). The RF locations and sizes of these RGCs suggested they form a mosaic that tiles space, despite their relatively sparse sampling (Figure 24 e1, e2). These cells were all selective for horizontal orientation, so we call them horizontal orientation (Figure 25 b) selective (HOS)-RGCs.

Figure 24: HOS RGCs from recording 1. a1: Temporal RFs. b1: Mean ISI (0.1 a.u., 10ms). c1: Average response to light steps (0.2 a.u., 2s). d1: Red: HOS RGCs (6), blue: other OFF RGCs (33). e1: RF mosaics. a2-e2: HOS RGCs (11) from recording 2.
The HOS-RGCs had the slowest and most biphasic temporal RFs out of all the OFF cell types (Figure 24 a1, a2). Their responses to the full field light steps were very transient (Figure 24 c1, c2). In fact, they seemed to fire to both increments and decrements of light intensity, and could potentially be ON-OFF cells (Figure 24 c1, c2). These cells also had the smallest RF diameters (Figure 24 e1, e2). Inspection of their spatial RFs revealed that their OFF center was flanked by a very strong inhibitory ON surround (Fig 25 a).

![Example Horizontal-OS RGC](image)

**Example Horizontal-OS RGC**

- **spatial RF**
- **drifting grating response**

![Example Vertical-OS RGC](image)

**Example Vertical-OS RGC**

- **spatial RF**
- **drifting grating response**

Figure 25: a. Spatial RF of one HOS RGC. b. Drifting grating response of HOS RGC to 8 directions of drifting grating stimulus (speed 150 μm/s). Each direction has rasters from 8 trials. middle: polar plot (blue) and vector sum (red). c-d: same as a-b for VOS RGC.

### 3.2.4.7 OFF vertical orientation selective RGCs

The second OS type had speckled ON and OFF regions in its spatial RF, non-Gaussian spatial RFs (Figure 25 c), and sustained ISIs (Figure 26 b1, b2). Parameters from the spatial RF contrast, spatial RF shape, and shape of the ISI distribution were utilized to classify this cell type (Figure 26 d1, d2). These cells were sparsely sampled by the MEA, but collective spatial RFs provided evidence for a mosaic arrangement (Figure 26...
e1, e2). Additionally, they all had temporal RFs that were almost triphasic (Figure 26 a1, a2), and were selective for vertical orientations (Figure 25 d), so we call them vertical orientation selective (VOS)-RGCs. The VOS-RGCs were different from the HOS-RGCs in terms of their spatial RF size, temporal RF structure, ISIs, and responses to full field light steps. We compare the functional properties of the 2 OS types in detail in Chapter 4.

![Population Properties of Vertical-OS RGCs](image)

Figure 26: VOS RGCs from recording 1. a1: Temporal RFs. b1: Mean ISI (0.1 a.u., 10ms). c1: Average response to light steps (0.1 a.u., 2s). d1: Red: VOS RGCs (4), blue: other OFF RGCs. e1: RF mosaics. a2-e2: VOS RGCs (3) from recording 2.

3.2.4.8 OS-RGCs and the orientation selective index

Neither the HOS nor VOS-RGCs were classified here by their orientation tuning (Figure 24, 26 d1, d2). To establish whether all the HOS and VOS RGCs were OS, we computed the orientation selective index (OSI, see Methods). The OSI was computed based on previous studies, which have attempted to identify orientation tuned cells in the rodent retina and LGN. The OSI varies from 0 to 1, with 1 indicating strong
orientation tuning. Previous studies have generally chosen a threshold OSI of 0.3, with larger values indicating OS-RGCs (Zhao, et al., 2013; Chen, et al., 2014). We plotted the distribution of OSIs of all RGCs and found that an OSI threshold of 0.4-0.5 (Figure 27 a, b) accurately distinguished OS-RGC types from other RGCs. Additionally, the 2 OS types had OSIs that were significantly higher than the other types (Figure 27 b, d, p value = 2e-6). This indicated that the HOS and VOS RGCs were strongly orientation selective.

**OSI distribution**

![Graphs showing OSI distribution](image)

**Figure 27:** a. Distribution of OSIs over 3 recordings (n = 802, includes cells identified only using white noise and gratings). b. Mean OSI for each RGC type. Black line is +/- 1 std. error of OSI. c-d: Same as a-b but for one recording (n = 241).
3.3 *Discussion*

The classification approach presented here identified 13 RGC types in the rat: six types were ON or OFF cells, four types were direction selective, two types were orientation selective, and one type had an expanding RF. Each type could be quantitatively and reproducibly distinguished from the other cells based on functional properties, demonstrating that RGC types at a given location are functionally distinct. The classification revealed 3 new aspects of visual information that were detected in the rat retina: stimulus expansion, horizontal orientations and vertical orientations. Additionally, the six types of ON and OFF cells showed that there were 3 distinct ON and OFF channels processing visual information at different spatial scales, temporal scales and spike rates. In the next chapter, we analyze the RF structure of each cell type, and provide additional insights into the computations performed by each type.

3.3.1 *Functional classification of rodent RGCs*

Classifying functional cell types quantitatively, reproducibly and verifiably is critical for many experiments, because it provides a method for comparing neural function across animals. This has at least two applications: comparing animals with constitutive genetic manipulations and comparing animals at different stages of disease progression. For neurodegenerative diseases like glaucoma, this will allow early detection (Della Santina, et al., 2013). It may also be useful for comparing neural function across species, and identifying how the visual environment and ecological niche of an
animal changes the computations performed by the retina (Banks, et al., 2015; Collins, 1999).

We followed the unsupervised classification approach adopted by most previous functional classifications of RGCs (Carcieri, et al., 2003; Farrow & Masland, 2011; Baden, et al., 2016), with the following differences. The first significant difference was that RGCs were classified using data from individual recordings instead of pooling data across recordings. This reduced the impact of inter-experiment variability which can either blur distinctions between cell types or cause the identification of too many cell types. It also allowed for cross-validation of the parameter selection step; selection occurred in one data set and was validated on other recordings. Second, relevant response features that distinguished each type were identified before classification. This improved performance of the GMM because it produced well-separated clusters thereby minimizing misclassification rates. Only two or three features were selected at each classification step, which kept data requirements for classification relatively low. Third, the classification approach was serial: one RGC type was classified at each step. This mitigated ambiguity in choosing the right number of clusters because each step consisted of fitting just two clusters. Finally, because many RGCs were recorded in each experiment, this allowed the mosaic arrangement of RFs to provide complementary evidence that the clustered cells were an irreducible type, and likely corresponded to a morphological type (Wassle, et al., 1981; Cook, 1998; Wassle, 2004).
While this approach was reproducible across recordings, it did not classify all recorded cells or identify all of the functional types. First, given an RGC density of 2000 cells/mm\(^2\), only ~10% of RGCs over the electrode array had well-isolated spikes. Second, only 63% (548 of 876) of the RGCs recorded across the three preparations were classified. 37% were not classified, either because too few cells of other types were sampled, or the stimuli used here did not reliably drive these cells to spike. Each stimulus used in this study was presented “full field”, which likely attenuated or silenced spiking in at least some RGC types e.g. local-edge detectors (van Wyk, et al., 2006; Zhang, et al., 2012). Third, only 13 RGC types were identified. This falls short of the 20-30 functionally distinct types that likely exist in most mammalian retinas (Kolb, et al., 1981; Volgyi, et al., 2009; Sanes & Masland, 2015; Baden, et al., 2016). Improvements in classification methods, using a wider variety of stimuli, and development of approaches for recording a higher fraction of RGCs simultaneously (Baden, et al., 2016) and over large retinal areas are needed for a complete and comprehensive functional classification of RGC types.

### 3.3.2 Correspondences to morphologically defined RGC types

A major goal in retinal research is to generate a complete catalog of RGCs that specifies the relationships between their functional and morphological types. We did not determine the morphology of the recorded RGCs; however their RF sizes and response kinetics provide some plausible correspondences. ON and OFF sustained RGCs likely
have large dendritic fields (corresponding to their large RFs) and large cell bodies because they produced large spikes on the electrode array. Both types exhibited deviations from a Gaussian RF structure, suggesting their dendrites may branch sparsely. Their sustained response kinetics suggests their dendrites ramify in the outer portion of the IPL (Borghuis, et al., 2014). These factors suggest a correspondence to the ON delta and OFF alpha cells, respectively of Peichl 1989. Similarly, the ON and OFF brisk transient cells may be the ON alpha and OFF delta cells from the same study (Peichl, 1989). The ON and OFF sluggish transient cells likely have smaller cell bodies and dendrites, suggesting correspondences to the outer and inner B1 RGCs (Huxlin & Goodchild, 1997). Cells sensitive to “looming objects” have been described functionally and morphologically in mouse retina (PV-5 RGCs; Munch, et al., 2009). Based on this work, the OFF expanding RGCs described here may correspond to C2 (outer) cells described by Sun et al (Sun, et al., 2002a). The morphological identities of the HOS-RGCs and VOS-RGCs are more opaque. Based on studies in rabbit, we expect these cells to stratify in the outer portion of the IPL and exhibit densely branching dendrites (Amthor, et al., 1989; Venkataramani & Taylor, 2010).

3.3.3 OS in the retina

Orientation Selectivity has been broadly thought of as arising in primary visual cortex (Hubel & Wiesel, 1962; Dhande, et al., 2015; Sun, et al., 2016), despite many reports of vertical and horizontal OS-RGCs (Levick, 1967; Levick & Thibos, 1982;
Amthor, et al., 1989; Venkataramani & Taylor, 2010; Nath & Schwartz, 2016). While this discrepancy is likely due in part to species differences, there is a growing appreciation that at least within the rodent visual system, orientation selective RGCs likely contribute to cortical orientation selectivity (Zhao, et al., 2013; Chen, et al., 2014). We found 2 types of OS-RGCs that are sharply tuned to horizontal and vertical orientations. Each OS-type tiled space regularly, providing a complete and independent sampling of horizontal and vertical orientations. While there is some evidence that other types of OS-RGCs are present in the rodent retina (Heine & Passaglia, 2011; Baden, et al., 2016), this indicates that cardinal orientations are represented preferentially. This is unlike OS cells in primary visual cortex, where selectivity is more balanced across orientations (Coppola & White, 2004; Van Hooser, et al., 2005; Zhao, et al., 2013; Kondo & Ohki, 2016). Thus, horizontal and vertical orientations are visual features that are robustly and explicitly computed in the rodent retina, and could contribute to the orientation tuning of neurons in central visual structures (Paik & Ringach, 2011; Zhao, et al., 2013).
4. Spatiotemporal RF diversity of rat RGC types

4.1 Introduction

The receptive fields (RFs) of retinal ganglion cells (RGCs) summarize the processing of visual information by the retina. The spatial and temporal structures of RFs define the features of the visual scene that are transmitted to the brain (Barlow, 1953; Kuffler, 1953; Gollisch & Meister, 2008). While the RFs of a few major RGC types in cats and primates have been examined extensively (Rodieck & Stone, 1965; Shapley & Victor, 1978; Kaplan & Shapley, 1986; Shapley & Perry, 1986; Dacey & Lee, 1994; Croner & Kaplan, 1995), the RF structure of most rodent RGC types is less well understood.

Rodents have become a major model system for basic and translational vision research, which necessitates a quantitative understanding of RFs throughout their visual system. This understanding, in turn, relies on a systematic classification of RGC types, because different types exhibit distinct RF structures.

In Chapter 3, we presented a robust classification method that classified multiple RGC types across preparations. In this Chapter, we quantitatively determine the RF structure of nine of those RGC types. Determining the RF structure of RGC types is critical to understanding the aspects of visual information processed at the level of the retina and identifying the signals transmitted to retinal recipient areas such as the lateral geniculate nucleus and superior colliculus. It can also define the range and complexities of computations performed by retinal circuits and provide insight into the presynaptic
mechanisms that give rise to these computations. We address three questions in this
Chapter: a) What are the computations performed by the different RGC types and what
does this convey about visual processing in the retina? b) How do the spatial and
temporal integration properties differ across RGC types? c) How do the spatial and
temporal integration properties of RGC types in the rat compare to those of other
mammals? This in turn provided several novel insights into visual processing in the
rodent retina.

To address these questions we carried out a systematic and quantitative
comparison of the spatial and temporal RF properties of nine cell types. We also used
singular value decomposition to determine the space-time separability of RFs, and
gained a new appreciation for what the different ranks of the RF convey about the visual
scene. This approach led to a collection of distinctive and fundamental findings about
RF organization among RGCs that have not been described previously in the rodent.

First, the rodent retina contains at least three pairs of ON and OFF cells that are
nearly symmetric in their response properties. These ON and OFF cell pairs partition the
encoding of spatiotemporal stimuli in a manner distinct from cats and primates (Troy &
Robson, 1992; Chichilnisky & Kalmar, 2002): RGCs with smaller spatial integration
exhibit more biphasic temporal integration, and pairs of ON and OFF RGCs exhibit
contrasting asymmetries in their RF structure. This indicates a distinctive functional
organization to the rodent retina.
Second, we observed that all nine RGC types exhibited RFs that were not independent in space and time (Enroth-Cugell, et al., 1983). These departures from independence arose from several distinct causes across types, and collectively indicate that linear integration of visual signals is incompletely captured by a single pair of spatial and temporal filters.

Third, we showed that the two OS types had functionally asymmetric RFs. Specifically, HOS-RGCs had smaller spatial RFs, prolonged temporal integrations and a linear RF that predicted their orientation tuning. This indicates that horizontal and vertical OS are encoded at different spatiotemporal scales and acquired through different mechanisms (Venkataramani & Taylor, 2010).

Collectively, these results provided several novel insights into visual processing in the rodent retina. They also provided a new understanding of the computations performed by distinct RGC types, and how that is distinct in several ways from other mammals.

4.2 Results

We determined the RF structure of the nine non-DS-RGC types. Low signal to noise in the spatiotemporal RFs of DS-RGCs precluded analyzing their RF structures. First, we provide a quantitative comparison of the RF structure across ON RGC types, OFF RGC types and OS types. We also compare the RF properties of these cell types to RGC types from other mammals. This comparison provided several insights into
common and distinct properties of each type and revealed differences in retinal organization from those described previously in cat and primates (Cleland & Levick, 1974; Rockhill, et al., 2002; Wassle, 2004; Gauthier, et al., 2009b). Second, we use SVD to determine the complexity of the RF structure of the different types, and gain additional insights into the computations they perform.

4.2.1 Spatiotemporal RF structure and rectification of ON RGC types

4.2.1.1 Spatial RF shape

Retinal RFs are often described as having a Gaussian shape (Devries & Baylor, 1997). However, the spatial RFs of individual ON brisk sustained cells exhibited complex shapes and jagged edges that deviated from a Gaussian (Figure 28a). The degree to which the different ON types had regular, compact spatial RFs was quantified by a spatial regularity index (SRI; see Materials and Methods). The SRI is a measure of how well the RF is fit by a convex hull. Gaussian RFs will have a convex hull that covers nearly the entire spatial RF (Figure 28b, c) without any gaps in between; more complex non-Gaussian RFs will have a convex hull that encompasses the spatial RF, but with gaps in between. The SRI is near one for cells with Gaussian-shaped RFs and substantially smaller than one for cells with complex non-Gaussian RFs.

As a population, ON brisk sustained RGCs exhibited a SRI distribution that was distinct from the 2 ON types (Figure 28d). The SRI of ON brisk sustained cells had a lower mean compared to the other 2 types. This analysis revealed that not all RGC types
have Gaussian RFs. Some types have more complex shaped RFs, which could be
indicative of the morphology of these cell types: they might have larger and more
sparsely branching dendritic fields than the other types.

4.2.1.2 Spatial RF overlap

Cell types in the retina sample visual space by forming RF mosaics. The spatial
RFs of cells overlap with their neighbors, which can result in some degree of signal
redundancy (Peichl & Wassle, 1979; Devries & Baylor, 1997; Segev, et al., 2006; van Wyk,
et al., 2006). A larger RF overlap between neighboring RFs implies greater signal
redundancy (Segev, et al., 2006; Borghuis, et al., 2008; Gauthier, et al., 2009b). Previous
work in the primate retina indicates that midget and parasol RGCs exhibit nearly
identical spatial RF overlap, despite differences in their temporal and spatial integration
(Gauthier, et al., 2009b). Studies in the rabbit retina also revealed that different cell types
exhibit nearly identical overlap (Devries & Baylor, 1997, Borghuis, et al., 2008). It is
unclear if cell types in the rodent retina follow a similar pattern. Comparing RF overlap
between mosaics of brisk sustained, brisk transient and sluggish transient RGCs
revealed differing degrees of overlap between each cell type (Figure 28 e). ON brisk
sustained cells had the largest degree of overlap, followed by the brisk transient and the
sluggish transient cells, reflecting their distinct roles in visual function (Gauthier, et al.,
2009b). These observations contrast with the findings in primate and rabbit, and indicate
that different RGC types in the rodent can exhibit different amounts of RF overlap and
signal redundancy across the population. These differences could be due to anatomical differences in dendritic overlap between cell types (Watanabe & Rodieck, 1989; Dacey & Brace, 1992; Dacey & Petersen, 1992) or a result of the functional differences between each type and the aspects of the visual scene each type is processing. Thus, encoding visual information with a uniform degree of redundancy is not a common organizing principle of the retina.

4.2.1.3 Space-Time integration

In primate and cat retina, RGCs with small spatial RFs (e.g. midget and beta cells respectively) exhibit sustained responses to light steps and weakly biphasic temporal filtering. In contrast, RGCs with larger spatial RFs exhibit transient responses to light steps and strongly biphasic temporal filtering (e.g. parasol and alpha cells; Cleland & Levick, 1974; Rockhill, et al., 2002; Wassle, 2004). We tested if this was a fundamental organizing principle of mammalian retinas. We found that the rodent retina exhibits a distinct organization. Specifically, ON brisk sustained RGCs had the largest spatial RFs (Figure 28a) while exhibiting the most sustained responses to full-field steps and weakly biphasic temporal RFs (Figure 28f). ON sluggish transient cells had the smallest spatial RFs (Figure 28c), transient responses to full-field light steps and the most biphasic temporal RFs (Figure 28f). Thus, spatial RF size and biphasic temporal integration are anti-correlated in the rodent retina (corr-coeff -0.98; Figure 28g).
4.2.1.4 Rectification in spike output

The relationship between stimulus and spike rate is one important factor in quantifying RGC function; it can be summarized by a nonlinear rectifying function, which specifies the mean spike rate given the degree of correlation between the stimulus and the spatiotemporal RF (Chichilnisky, 2001). Each ON type exhibited a distinct rectifying function (Figure 28 h). ON brisk sustained cells exhibited the highest gain and the highest firing rate at zero contrast (x axis), while ON brisk transient and sluggish transient cells exhibited progressively less gain and lower firing rates (Figure 28 h). Across ON types, a more biphasic temporal RF correlated with (smaller) rectification in the gain function (corr-coeff -0.97; Figure 28 i). The 3 ON types are therefore very distinct in their RF structure and rectification, and how they process visual information.

**Spatiotemporal RF properties and Rectification of 3 ON Types**

![diagram](image)

Figure 28: a-c. Spatial RFs of 2 e.g. RGCs of each ON type. d. Mean and dist. of SRI for each type (77 BS, 104 BT, 30 ST). e. RF profiles. f. Average Temporal RFs (0.15 a.u., 60ms). g. Space-Time corr. h. Average SNLs. i. SNL-Time corr. Error bar is 1SEM.
4.2.2 Spatiotemporal RF structure and rectification of OFF RGC types

The functional organization of the OFF brisk sustained, brisk transient and sluggish transient RGC types was highly consistent with that of the three ON types, indicating a conserved organization to ON and OFF processing in the rodent retina.

4.2.2.1 Spatial RF shape

The RF shapes of OFF brisk sustained cells also appeared to be more irregular than the other OFF cell types (Figure 29 a, b, c). To determine if the OFF brisk sustained type exhibited irregular spatial RF shapes, the RFs were quantified by the SRI. A comparison of the SRIs across cells of each type revealed a picture that was similar to the ON RGCs: OFF brisk sustained cells exhibited a distribution of SRIs that was distinct from the other two types. The SRIs of OFF brisk sustained cells had a lower mean compared to the brisk transient and sluggish transient cells (Figure 29 d). Thus, OFF brisk sustained cells were similar to ON brisk sustained cells in terms of their spatial RF shape: they exhibited more irregular shaped RFs. This might be indicative of the morphology of these cell types: they might have larger and more sparsely branching dendritic fields than the other types.

4.2.2.2 Spatial RF overlap

The amount of RF overlap between mosaics of brisk sustained, brisk transient and sluggish transient RGCs were compared. Surprisingly, in contrast to the ON RGCs, the three OFF RGC types exhibited nearly identical overlap among neighboring spatial
RFs (Figure 29 e). The RF overlap was similar to that observed previously among OFF parasol and OFF midget cells in the primate retina (Gauthier, et al., 2009b). Hence, OFF types with distinct functional properties exhibited uniform signal redundancy, while ON types exhibited variable signal redundancy. This finding is distinct from that of primates, where both ON and OFF types had uniform signal redundancy. This suggests that there is some degree of functional asymmetries between ON and OFF cell types in the rodent retina, in terms of how they sample space. These differences could be due to anatomical differences in dendritic overlap between ON and OFF cell types: for example, the dendritic fields of ON alpha cells in the rat are larger than their OFF counterparts (Peichl, et al., 1987; Peichl, 1989; Tauchi, et al., 1992). It could also be a result of how ON vs OFF stimuli need to be represented and sampled in the rodent retina.

4.2.2.3 Space-time integration

Similar to the ON RGCs, OFF brisk sustained RGCs had the largest spatial RFs (Figure 29 a), exhibited the most sustained responses, and had the least biphasic temporal RFs (Figure 29 f, g; corr-coeff -0.98). OFF sluggish transient cells had the smallest spatial RFs (Figure 29 c), most transient responses to full-field steps and the most biphasic temporal RFs (Figure 29 f). Therefore the partitioning of visual stimuli into different spatiotemporal frequency bands is relatively symmetric between ON and OFF pathways in the rodent retina, while differing from that in the cat and primate.
4.2.2.4 Rectification in spike output

The nonlinear rectifying functions of OFF cells were also ordered similarly to the ON cells (Figure 29 h). OFF brisk sustained cells had the steepest functions and exhibited the highest spike rates at zero contrast (x axis = 0). OFF brisk transient cells exhibited less gain and lower firing rates than brisk sustained cells, and OFF sluggish transient cells displayed the lowest gain and firing rates (corr-coef -0.99; Figure 29 h). Across OFF RGC types, a more biphasic temporal RF correlated with (smaller) rectification in the gain function (corr-coef -0.97; Figure 29 i). The 3 OFF types were therefore distinct in their RF structure and rectification, nearly functionally symmetric with the 3 ON types and had a distinct functional organization from that found in cats and primates.

Figure 29: a-c. Spatial RFs of 2 e.g. RGCs of each OFF type. d. Mean and dist. of SRI for each type (89 BS, 133 BT, 28 ST). e. RF profiles. f. Average Temporal RFs (0.15 a.u., 60ms). g. Space-Time corr. h. Average SNLs. i. SNL-Time corr. Error bar is 1SEM.
4.2.3 Spatiotemporal RF structure of OS-RGC types

HOS and VOS RGCs encode the same visual feature: orientation. However, in Chapter 3, it was shown that the 2 OS types differed in terms of their functional properties. Previous studies of HOS and VOS RGCs in the rabbit have demonstrated that the synaptic mechanisms that generate OS in these two types are different (Venkataramani & Taylor, 2010). We tested whether the spatiotemporal RF properties of these 2 OS types were also distinct. To test this, the diameter of the spatial RFs of both cells types and the duration of temporal integration was measured. Clear asymmetries were present between the spatial and temporal structures of HOS-RGCs and VOS-RGCs (Figure 30 a). VOS-RGCs had larger spatial RFs (RF diameter: 422 +/- 63 microns) and briefer temporal RFs (t-zero: 209 +/- 6 ms), while HOS-RGCs had smaller spatial RFs (RF diameter: 267 +/- 40 microns) and longer temporal RFs (t-zero: 188 +/- 5 ms) (Figure 30 a). This indicates that vertical and horizontal orientations are encoded at different spatiotemporal scales in the rodent retina.

Figure 30: a: Mean, standard error and distribution of RF diameters and time of zero crossing for HOS (n = 30) and VOS (n = 11) RGCs from all 3 recordings. b: RF mosaic and Spatial RFs of all HOS RGCs from one recording. c: Same as b for VOS-RGCs.
Furthermore, upon inspection of the spatial RFs of OS-RGCs, we noticed that the spatial RFs of HOS-RGCs exhibited an asymmetric surround that was localized to regions superior and inferior to the center, but absent along the orthogonal axis (Figure 30 b). In contrast, VOS-RGCs had spatial RFs that did not exhibit a clear distinction between the center and surround, but had a “speckled” quality with ON and OFF sub regions (Figure 30 c). We tested whether the spatial RF could predict the OS of each cell type. A spatial RF that predicts the orientation tuning could potentially imply that a simple mechanism gives rise to OS, while a spatial RF that does not predict the orientation tuning implies that a more complex mechanism gives rise to OS.

To test whether the spatial RF could predict the OS, the dot product of a stationary drifting grating stimulus to the spatial RF at 8 different orientations was computed. This generated a predicted direction tuning curve for each cell. The predicted tuning curves were averaged together across all cells of a type (blue, Figure 31 b, d), and compared to the average actual tuning curves (black, Figure 31 b, d). The prediction was a closer match to the actual tuning curves for HOS-RGCs (Figure 31 a) compared to VOS-RGCs (Figure 31 c). Thus, the asymmetric surround indicated that horizontal tuning could be predicted by the linear spatial RF structure of HOS-RGCs, while the lack of a clear surround indicated that orientation tuning could not be predicted from the linear RF of VOS-RGCs (Figure 31). These results indicate that in rodents, vertical and horizontal orientations are encoded at different spatiotemporal scales and by different
RF structures: horizontal orientations are encoded by linear RFs; vertical orientations are encoded by nonlinear RFs.

Figure 31: a: Response of a HOS RGC to 8 directions and 8 trials of a drifting grating stimulus (speed 150 μm/s). middle: polar plot (blue) and vector sum (red). b: Average actual and predicted tuning curves of all HOS-RGCs. c-d: Same as a-b but for VOS-RGCs.

4.2.4. Comparison of ON and OFF RGCs: asymmetries

While comparing the ON and OFF brisk sustained, brisk transient and sluggish transient cell types above, we noticed that they were functionally asymmetric in terms of how they sampled visual space. Previous studies in primates and other mammals have noted functional asymmetries in the RFs of ON and OFF cells (Chichilnisky & Kalmar, 2002; Zaghloul, et al., 2003). For example, ON parasol and midget RGCs in primates exhibit systematically larger spatial RFs than OFF parasol and midget RGCs. These asymmetries between ON and OFF pathways have been implicated as being optimal given the structure of natural scenes (Ratliff, et al., 2010). We tested whether functional asymmetries existed between ON and OFF types in the rodent retina, and if functional
organizational principles like systematic asymmetry could be generalized across species. We show that the rodent retina exhibits a distinct functional organization.

4.2.4.1 RF size

OFF RGCs did not exhibit consistently smaller spatial RFs than their ON counterparts. Only ON brisk sustained RGCs exhibited larger spatial RFs than their OFF-type counterparts (Figure 32 a). The results were consistent when using a nonparametric estimate of RF size (see Materials and Methods). This could suggest differences in morphology between the ON and OFF types such as the ON brisk sustained RGCs having a larger dendritic field size than their OFF counterparts.

4.2.4.2 Temporal integration

Among brisk sustained RGCs, OFF cells had slower temporal integration than ON cells (Figure 32 b, blue). This relationship was reversed for brisk transient RGCs (Figure 32 b, red). Therefore, the dynamics of temporal integration was determined less by ON vs. OFF and more by RF size: cells with larger spatial RFs (Figure 32 a), exhibit briefer temporal integration.

4.2.4.3 Contrast gain functions

While spatial and temporal RFs did not exhibit consistent asymmetries between pairs of ON and OFF cells, contrast gain functions were consistently steeper among OFF cells than ON (Figure 32 c; Chichilnisky & Kalmar, 2002). There was no consistent trend across ON and OFF types for an offset in this contrast gain function (Figure 32 d).
Therefore, OFF RGC types consistently exhibited higher gain than ON cells, but did not exhibit greater rectification.

Hence, we conclude that functional asymmetries exist between ON and OFF types in the rodent; however these were not systematic like that found in primates. The functional organizational principles could not be generalized to the rodent. These differences in retinal organization may reflect differences in the organization of visual processing in downstream areas such as primary visual cortex (Laramee & Boire, 2015). Furthermore, the physical constraints imposed by the small and lateralized eyes in rodents may produce differences in retinal organization compared to species with larger eyes and more binocular vision (Collins, 1999; Huberman & Niell, 2011).

**Figure 32:** a-d: Functional asymmetries of ON and OFF types. Each point shows the mean of the parameter for all ON and OFF BS (blue), ON and OFF BT (red) and ON and OFF ST (green) from one recording. Error bars are 1SEM. 461 total RGCs from 3 recordings shown.
4.2.5. Spatiotemporal RF complexity of RGC types

The previous analyses compare the spatial and temporal structure of RFs independently. This is a common assumption made when analyzing cell types in the retina, because it is a simple way to characterize cell types and is useful in terms of investigating their functional properties (Rodieck & Stone, 1965; Gauthier, et al., 2009a). Analyzing the entire spatiotemporal structure of the RF, instead of just its separable space and time components, might allow us to further understand and appreciate any additional computations performed by cell types.

In this section, we determined the space-time separability of nine cell types. We used space-time plots to visualize the RFs, and SVD to provide additional insight into the computations performed by each type. Space time plots provide a compact way to visualize changes in the spatial RF over time. The 3D spatiotemporal RFs of all cells within a type were averaged together, and collapsed along one spatial axis, to get a 2D space-time plot (Figure 33). For a perfectly space-time separable RF, the size of the spatial dimension remains constant over time. For an inseparable space-time RF, the spatial RF will change over time, and the space-time plot will exhibit some diagonal structure. Space-time plots of the RGC types (Figure 33) exhibited some degree of diagonal structure. Most cell types showed a weak diagonal structure, while the OFF expanding (Figure 33 g) and the OFF Vertical OS type (Figure 33 i) displayed strong
diagonal structures. This suggested that all types deviated from space-time independence (DeAngelis, et al., 1993).

\[ \text{Space-Time Plots for RGC types: } y \text{ vs } t \]

To quantify these changes, singular value decomposition (SVD) was performed on all the cells of each type. SVD provides a framework for characterizing the extent of deviations from space-time independence (Stewart, 1993; Depireux, et al., 2001; Emerson & Vaughn, 2011). SVD yields an ordered set of filters that capture progressively less variance in the RF, and a spectrum of eigenvalues that quantify the relative contribution
of these filters to the total RF. For RFs that are space-time independent, SVD yields a single pair of significant filters: one filter for the spatial RF and another for the temporal RF. Subsequent filters and their weights arise from noise in the measured RF. For RFs that are space-time dependent, SVD yields more than one significant pair of filters, where the number of significant filters can be determined via bootstrapping (Methods). Analyzing the structure of the additional filters can provide further insights into the computations performed by each type.

Figure 34 shows the mean variance of each filter for each cell type. The variance of the filter was computed for each cell within a type, averaged together, and the means and standard errors were plotted. On the right are the average temporal filters associated with the first three significant kernels (Figure 34). It can be seen that all cells of a type displayed very similar space-time dependencies. Figure 35 shows the variance of the space-time filters for an example cell from each type. Each point represents a filter, and all points above the red line are significant filters. The plots on the right are the spatial and temporal kernels associated with the first three significant filters.
Figure 34: Variance explained by 1st 20 filters for each RGC type. All cells of a type were averaged together and the mean and SEM of each filter was plotted. a: ON BS RGCs. b: ON BT. c: ON ST. d: OFF BS. e: OFF BT. f: OFF ST. g: OFF Exp. h: OFF HOS. i: OFF VOS.
4.2.5.1 Space-Time dependence of ON brisk sustained, brisk transient and sluggish transient RGCs

SVD applied to the three ON RGC types revealed small deviations from space-time independence. For all three types, the first pair of filters captured the majority of the variance in the STA. The first filter captured on average 89% of the variance for brisk sustained cells (Figure 34 a), 96% of the variance for brisk transient cells (Figure 34 b), and 93% of the variance for sluggish transient cells (Figure 34 c). The second filter captured on average 4% of the variance for brisk sustained cells, 1% of the variance for
brisk transient cells, and 2% of the variance for sluggish transient cells (Figure 34 a-c).

Even though the variance captured by the second filter pair was small, it was statistically significant in all 3 ON types (one example shown, Figure 35 a, c, e).

To gain additional insight into the computations performed by these cell types, we analyzed the spatial and temporal structure of the second filter pair. We observed that the structure of the rank-two spatial filter for all 3 ON types was concentrated in the RF surround (Figure 35 b, d, f: pair 2), and the associated rank-two temporal filters peaked earlier than the rank-one filters (Figure 35 b, d, f). These features indicated that the rank-two filters reflected a RF surround that integrates signals with kinetics distinct from that of the RF center (Enroth-Cugell, et al., 1983).

We tested this by explicitly examining the temporal dynamics of the RF center and surround of each RGC type and comparing that to the first two temporal filters obtained by SVD. To obtain the kinetics of the RF center and surround, we took two horizontal cuts of the space time plot (over time). The first cut was through a pixel in the RF center with the highest pixel intensity, and the second cut was through a pixel in the RF surround with the highest intensity (Figure 33 a-c). Since the space-time plot was an averaged RF, we also compared the dynamics of this averaged RF to the temporal dynamics of individual pixels with the highest intensity in the center and surround.

The center and surround temporal filters from the spatiotemporal RF coincided exactly with the first and second temporal filters from SVD. Hence, SVD revealed
distinct temporal integration between the RF center (black, Figure 36 a-c) and surround (red, Figure 36 a-c). All three types displayed a difference in the peak time between center and surround, with ON brisk sustained RGCs exhibiting the largest difference (Figure 36 a). This showed that all the ON types exhibited a time lag between the center and surround, with the surround peaking before the center.

**Figure 36:** Kinetics of the center (black) and surround (red), averaged across all RGCs of a type. Center and Surround temporal kinetics are normalized to be on the same scale. a: ON BS. b: ON BT. c: ON ST. d: OFF BS. e: OFF BT. f: OFF ST.

4.2.5.2 Space-Time dependence of OFF brisk sustained, brisk transient and sluggish transient RGCs

The 3 OFF RGC types also exhibited small deviations from space-time independence, similar to the 3 ON RGC types (Figure 34 d-f, 35 g-l). For all three types, the first pair of filters captured the majority of the variance in the STA (Figure 34 d-f, Figure 35 g, i, k). The first filter captured on average 93% of the variance for brisk sustained cells (Figure 34 d), 97% of the variance for brisk transient cells (Figure 34 e), and 88% of the variance for sluggish transient cells (Figure 34 f). The second filter captured on average 3% of the variance for brisk sustained cells, 1% of the variance for brisk transient cells, and 3% of the variance for sluggish transient cells (Figure 34 d-f).
Again, even though the variance captured by the second filter pair was small, it was statistically significant in all 3 OFF types (Figure 35 g, i, k, one example cell shown).

We observed that the structure of the rank-two spatial filter for all 3 OFF types was also concentrated in the RF surround (Figure 35 h, j, l: pair 2), and the associated temporal filters peaked earlier than the rank-one filters (Figure 35, h, j, l). Again, we tested this by comparing the temporal dynamics of the RF center and surround of each RGC type to the first two temporal filters obtained by SVD (Figure 36 d-f). The center and surround temporal filters from the spatiotemporal RF (Figure 36 d-f) coincided exactly with the first and second temporal filters from SVD (Figure 35 h, j, l). All three types displayed a difference in the peak time between center and surround, with OFF brisk sustained RGCs exhibiting the largest difference (Figure 36 d). This showed that all the three OFF types had a RF surround that integrated signals with kinetics distinct from that of the RF center.

4.2.5.3 Space-Time dependence of OFF expanding RGCs

The space-time plot of the Off Expanding cell type revealed a diagonal structure, indicating that the spatial RF expanded in time (Figure 33 g). This expansion could also be observed directly by analyzing sequential frames from the STAs of individual cells (Figure 37 a, top row) and from the STAs averaged across the population of cells (Figure 37 b, top row). Expansion of this amount was not observed in other cell types (Figure 37 a, b, bottom row). To confirm the expanding nature of the spatial RFs of the
Expanding cells, and establish that it was distinct from the other types, we computed the average RF area of each type over five frames preceding a spike. On average, the RF area of the Expanding cells increased by 39% over the three frames preceding a spike (Figure 37 c) and by 110% over five frames preceding a spike (Figure 37 b, top row). Other cell types expanded by at most 13% (Figure 37 c). The rate of expansion in the RF suggested that these cells were tuned for stimuli expanding at a rate of 2.4 mm/s on the retina.

Figure 37: a. top (bottom): Spatial RF expansion across 5 frames of the STA for an example OFF Exp (OFF ST) RGC. b. Same as a, but averaged across all OFF Exp and ST RGCs in one recording. c. Normalized average RF area across 3 frames of the STA for each type.

SVD further confirmed the complex space-time RF structure among OFF expanding RGCs (Figure 34 g, 35 m, n). Their RFs were composed of 3 to 4 significant filter pairs (Figure 34 g, 35 m). The first space-time filter pair accounted for 61% of the RF variance, while the second pair accounted for 21%, on average (Figure 34 g). This analysis demonstrates these RFs deviated substantially from space-time independence. Unlike the 3 ON and OFF types, we were unable to determine the exact nature of the computation carried out by the second filter. Nevertheless, we can still conclude that the expanding nature of the RF gave rise to the space-time dependency of this cell type.
4.2.5.4 Space-Time dependence of OFF HOS and VOS RGCs

The space-time plots of HOS and VOS RGC types were very distinct, with VOS-RGCs exhibiting strong space-time dependence (Figure 33 i) and HOS-RGCs exhibiting weaker space-time dependence (Figure 33 h). This indicates that HOS and VOS RGCs have very different RF structures, and could potentially employ different mechanisms to encode OS. SVD revealed further differences in their RF structures. HOS-RGCs had relatively separable RFs, with the first space-time filter pair explaining 91% of the RF variance, on average (Figure 34 h, 35 o). The first filter pair captured the center of the RF and a horizontally tuned surround, which could account for its orientation tuning (Figure 35 p). A second filter pair was also frequently significant and exhibited spatiotemporal structure that was consistent with a vertically tuned RF surround (Figure 35 p, o: inset, pair 2). SVD applied to the linear RFs of VOS-RGCs revealed larger deviations from space-time independence: the first space-time filter pair accounted for just 60% of the variance (Figure 34 i) and the cells typically exhibited 3 to 4 significant filter pairs (Figure 35 q). The first space-time filter captured speckled ON and OFF regions in the spatial RFs of these cells, while the second filter captured a more symmetric spatial RF (Figure 35 r). However, the origin of this space-time dependence and the computations performed by the significant space-time filters was unclear: it was not generated by a RF surround with distinct temporal filtering from the center, nor was it generated by expansion of the RF center in time. These results indicate that in rodents,
vertical and horizontal orientations are encoded by different RF structures: horizontal orientations are encoded by linear RFs that are relatively independent in space and time; vertical orientations are encoded by nonlinear, space-time dependent RFs.

4.3 Discussion

The RF measurements and comparisons across types revealed several key features of retinal organization. First, RGCs with small spatial RFs had more biphasic temporal RFs. Thus, higher spatial resolution pathways exhibit more band-pass temporal filtering. This is different from the organization observed in primate and cat retinas, suggesting different principles guide retinal organization in rodents. Second, no RGC type identified here exhibited a RF structure that was strictly independent in space and time. ON and OFF RGCs exhibited distinct temporal integration in the RF surround compared to the center while OFF expanding RGCs and VOS-RGCs exhibited large deviations from space-time independence. Third, the two OS RGC types were functionally asymmetric: they integrated visual signals at different spatiotemporal scales and acquired OS through different mechanisms. Below we discuss some limitations of this study, compare the ON and OFF RGC pairs to other mammals, and consider how the center-surround time lag could be influenced by presynaptic mechanisms.

4.3.1 RF structure

RFs identify the stimulus features that are encoded by neurons (Cleland & Levick, 1974; Chichilnisky, 2001; Keat, et al., 2001). A major aspect of RF structure is the
extent to which they are independent in space and time; space-time independence suggests relatively simple circuit mechanisms and simple stimulus features drive RGC spiking. Previous studies in cat indicated that X and Y cells exhibit small, but significant deviations from space-time independence caused by a temporal lag between center and surround (Enroth-Cugell, et al., 1983; Dawis, et al., 1984; Cai, et al., 1997). Consistent with these earlier studies, we found that no RGC type in rodent was strictly space-time independent. Most types exhibited distinct temporal filtering between the center and surround. However, we also observed more extreme examples of space-time dependencies such as spatial RFs that expand in time by as much as 100%. These cells may signal optic flow or looming (Munch, et al., 2009).

An important limitation in the RF measurements presented here is that they are linear estimates. These estimates have been shown in some circumstances to accurately capture the stimulus features that drive RGC spiking (Chichilnisky, 2001; Keat, et al., 2001; Pillow, et al., 2005). However, for some RGC types, stimulus features interact nonlinearly in space and time (Schwartz, et al., 2012). One example nonlinearity common to many RGCs are “subunits” within the spatial RF that have a rectified output (Hochstein & Shapley, 1976; Demb, et al., 2001; Schwartz, et al., 2012; Freeman, et al., 2015). Subunits and other RF nonlinearities are likely important for a complete understanding of at least some of the RGC types described here. For example, the orientation tuning of VOS-RGCs was not predicted from their linear RF, indicating that a
linear estimate of the RF failed to fully capture stimulus features that drive or suppress spiking. Thus, an important goal in future work will be to measure these nonlinearities and to determine their impact on shaping RF structure across RGC types.

4.3.2 ON and OFF cell functional relationships

Functional asymmetries between pairs of ON and OFF cell types exist in primate and guinea pig retinas (e.g. ON and OFF parasol cells; Chichilnisky & Kalmar, 2002; Zaghloul, et al., 2003). These asymmetries include OFF cells having smaller spatial RFs, more rectified gain functions, and briefer temporal integration (Chichilnisky & Kalmar, 2002). This organization may be optimal for encoding and transmitting information about natural scenes (Ratliff, et al., 2010). In this study, three pairs of ON and OFF cells were functionally similar: brisk sustained, brisk transient and small transient RGCs. Without the cell morphology, these pairings are best-guesses based on functional properties. Nevertheless, a comparison of their functional properties produces a view that differs in several respects from previous work. Some OFF RGC types had larger RFs and slower temporal integration than their counterpart ON cells. The only relationship identified here that was consistently different between functional pairs of ON and OFF cells was a steeper contrast gain function among OFF cells. Thus, the functional organization of ON and OFF pathways appears distinct in rodent retina compared to that observed in primate parasol and midget cell pathways.
4.3.3 Coarse-fine feature tuning in the rodent retina

In LGN and visual cortex, coarse features of an image are processed before finer features (Bredfeldt & Ringach, 2002; Allen & Freeman, 2006; Nirody, 2014). This “coarse-fine tuning” is thought to support a comparison between fine-scale structure and coarse-scale context, potentially supporting an efficient coding hypothesis (Allen & Freeman, 2006, Barlow, 1961).

The temporal delay between center and surround is one way in which coarse features are integrated earlier than fine features (Allen & Freeman, 2006). This time lag was observed in 6 ON and OFF RGC types, providing evidence that coarse-fine feature tuning begins in the retina and that many RGC types exhibit this tuning. A natural mechanism for this tuning is lateral inhibition from horizontal or amacrine cells. Lateral inhibition introduces a delay relative to feedforward excitation because signals must both traverse a lateral distance and an additional synapse. This will necessarily introduce a lag between the surround and center, whereby the surround response is generated by signals that occurred earlier than those that generated the center response. The observation that this tuning was prevalent across RGC types with otherwise distinct spatial and temporal integration in the RF center indicates it is a conserved feature of RF structure in the early visual system, across both parallel pathways and species.
5. Predicting the neural code of RGC types with the LN model

5.1 Introduction

RGCs encode visual stimuli in the form of spikes. These spikes are sent via the optic nerve to downstream visual areas, and convey information about the visual world to the rest of the brain. To completely understand what the retina processes and communicates to the brain, it is necessary to decipher what each spike signals about the visual world (Keat, et al., 2001). Deciphering the spiking output of neurons is important because it provides insights into the visual stimuli encoded by RGCs and the computations that RGCs perform (Berry, et al., 1997; Meister & Berry, 1999; Keat, et al., 2001). It can also aid in the development of better neural prosthetic devices that aim to replicate the spiking output or neural code of the retina, and recreate vision for blind patients (Nirenberg & Pandarinath, 2012).

In this Chapter, we study the neural code of the retina. The retina is a popular model to study the neural code because it offers a few advantages. First, it has a well-defined set of inputs (visual stimuli) and outputs (spiking responses of RGCs) which facilitates the design of experiments that study neural coding (He, et al., 2003; Wassle, 2004). The two-dimensional structure of the retina also allows the spiking responses of multiple RGCs, and therefore multiple neural codes, to be studied simultaneously, through the use of multi-electrode arrays (Meister, et al., 1994; Meister, 1996; Berry, et al., 1997). Additionally, the retina presents an opportunity to study a diverse set of...
neural codes. This is because as demonstrated in Chapters 4 and 5, there are numerous RGC types, each with its own unique neural code that signals distinct information about the visual scene to the brain.

To capture the neural code of RGCs, various computational models have been utilized (Meister & Berry, 1999; Keat, et al., 2001; Pillow, et al., 2005; Field & Chichilnisky, 2007). One example of a computational model is the Linear-Nonlinear (LN) model. The LN model consists of a linear filter, that is estimated using reverse correlation, and a nonlinear component that takes into account the spiking dynamics of the RGC. The LN model is popular because it is simple, mathematically tractable, and has been successful at predicting the spiking responses of neurons in the early visual system - it explains more than 70% of the variance in spiking activity of individual RGCs (Pillow, et al., 2005). However, it is unclear how well this computational model generalizes across distinct RGC types. It is important to evaluate the performance of this model across cell types, because each type has a unique neural code and encodes visual information in a distinct manner (Koch, et al., 2006). Evaluating how the model performs on each cell type will also allow us to gain an appreciation for additional computations that might be carried out by each cell type that are not captured by the spatiotemporal RF alone. Given the distinct aspects of visual information processed by each RGC type, this is likely to be the case, as certain types might carry out multi-dimensional nonlinear computations. Additionally, it provides insights into the cell types where a white noise stimulus does
not completely capture aspects of the visual world that excite the cell type, and initiates the search for other potential trigger features for these types.

In this Chapter, we predict the neural code signaled by the 13 different RGC types identified in Chapter 3. We use the Linear-Nonlinear Model to predict the neural code of each type. We utilize the LN model because it is simple, mathematically tractable, and has been successful at predicting the spiking responses of neurons in the early visual system (Chichilnisky, 2001; Pillow, et al., 2005; Schwartz, et al., 2006). We compare how well the LN model performs at predicting the spiking responses across the different RGC types. We find that the LN model performs better at predicting the spiking responses for RGC types with highly space-time independent RFs. However, it performs poorly when predicting the spiking responses of RGC types with highly space-time dependent RFs. The difference in performance of the LN model across cell types provides insights into the complexity of computations performed by each cell type and highlights the need for more complex models and stimuli to fully capture the spiking output of RGCs.

5.2 Results

To determine how well the LN model predicted the spiking activity of RGC types, multiple RGCs were simultaneously recorded using a MEA. The RGCs were shown four types of visual stimuli: white noise, drifting gratings, full field light steps and white noise repeats. Response properties from the white noise, drifting gratings and
full field light steps were used to classify the RGCs into 13 distinct types: 4 DS types, 3 ON types and 6 OFF types (described in detail in Chapters 3 and 4). Cells within each RGC type had very similar temporal RFs, and each RGC type was verified by checking for the presence of spatial RF tiling. All 13 RGC types were identified, and their firing patterns and firing rate predictions were analyzed in 3 different preparations. The firing patterns were consistent across preparations, and data from one preparation is presented below.

5.2.1 Firing rates across RGC types

5.2.1.1 Mean firing rates

First, the firing rates of different RGC types to both the white noise and white noise repeats stimulus were compared (Table 1). RGC types differed considerably in their firing rates, with the OFF brisk sustained RGCs having the highest mean firing rate to both the white noise and white noise repeats stimulus. The Off HOS-RGCs had the lowest firing rates to both stimuli. The OFF sluggish transient RGCs, DS-RGCs and expanding RGCs also had low firing rates. This suggested that these types either fired in a sparse manner or that the stimulus did not completely capture the aspects of the visual scene that excited these cell types.

Among the 3 ON types, the ON brisk sustained RGCs had the highest mean firing rates, followed by the ON brisk transient RGCs, and the ON sluggish transient RGCs (Table 1). This pattern was similar for the 3 OFF types: the OFF brisk sustained
RGCs had the highest mean firing rates, followed by the OFF brisk transient RGCs, and the OFF sluggish transient RGCs. This was consistent with the functional pairing observed between the ON and OFF types in Chapter 4. Cells with the largest RFs and least biphasic temporal integration had higher mean firing rates and cells with the smallest RFs and most biphasic temporal RFs had lower mean firing rates. This suggested that the three types were processing distinct aspects of the visual scene, and that larger stimuli were processed at faster time scales and higher firing rates, while smaller stimuli were processed at slower time scales and lower firing rates.

Also in line with the functional asymmetries observed across the 3 ON and OFF types, OFF brisk sustained and OFF brisk transient RGCs had higher firing rates than the ON brisk sustained and ON brisk transient RGCs. This was consistent with the differences in the slope of the SNL observed across cell types in Chapter 5, with the OFF brisk sustained and transient cells having a higher gain than the ON cells (Figure 33 c). The differences in firing rates across ON and OFF functional pairs implies that they are not truly parallel pathways, and could be driven by different presynaptic circuits (Zaghloul, et al., 2003).

Though the mean firing rates differed across RGC types, when comparing the mean firing rates across stimuli (white noise vs white noise repeats, Table 1: columns 1 and 2) we found that the firing rates were stable. For example, the OFF brisk sustained RGCs always had the highest firing rates, the OFF HOS-RGCs, DS-RGCs, OFF sluggish
transient and OFF expanding cells had the lowest firing rates and the relative symmetries and asymmetries in the firing rates for the 3 ON and OFF types were conserved across both stimuli (Table 1). This suggested that the cells fired in a reliable manner to both sets of white noise stimuli, and were stable as the experiment progressed. As the mean firing rates were stable, this allowed the LN model to be estimated from the white noise stimuli and tested on the white noise repeats stimuli.

### Table 1: Mean and peak firing rates of RGC types to white noise (WN) and white noise repeats stimulus.

<table>
<thead>
<tr>
<th>RGC Types</th>
<th>Mean Firing Rate to WN (Hz)</th>
<th>Mean Firing Rate to WN repeats (Hz)</th>
<th>Peak Firing Rate to WN repeats (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS</td>
<td>3.97 ± 1.16</td>
<td>3.34 ± 1.09</td>
<td>53.56 ± 17.04</td>
</tr>
<tr>
<td>On Brisk-Sust</td>
<td>23.16 ± 9.14</td>
<td>21.35 ± 5.80</td>
<td>65.52 ± 14.03</td>
</tr>
<tr>
<td>On Brisk-Trans</td>
<td>14.16 ± 1.46</td>
<td>12.98 ± 1.39</td>
<td>184.32 ± 17.37</td>
</tr>
<tr>
<td>On Sluggish-Trans</td>
<td>8.71 ± 2.08</td>
<td>7.32 ± 1.68</td>
<td>110.93 ± 12.94</td>
</tr>
<tr>
<td>Off Brisk-Sust</td>
<td>31.60 ± 7.07</td>
<td>35.35 ± 6.01</td>
<td>109.97 ± 9.52</td>
</tr>
<tr>
<td>Off Brisk-Trans</td>
<td>28.11 ± 5.05</td>
<td>23.18 ± 2.83</td>
<td>125.96 ± 9.30</td>
</tr>
<tr>
<td>Off Sluggish-Trans</td>
<td>3.96 ± 1.10</td>
<td>3.76 ± 0.96</td>
<td>98.57 ± 33.70</td>
</tr>
<tr>
<td>Off Expanding</td>
<td>4.27 ± 1.65</td>
<td>5.31 ± 2.17</td>
<td>84.24 ± 21.02</td>
</tr>
<tr>
<td>Off Hor-OS</td>
<td>2.58 ± 0.73</td>
<td>2.53 ± 0.68</td>
<td>34.70 ± 10.51</td>
</tr>
<tr>
<td>Off Vert-OS</td>
<td>8.30 ± 3.79</td>
<td>8.96 ± 3.81</td>
<td>67.89 ± 23.94</td>
</tr>
</tbody>
</table>

#### 5.2.1.2 Peak firing rates

Next, the peak firing rates were compared across cell types (Table 1: column 3).

The peak firing rates were significantly higher than the mean firing rates across all cell types. Interestingly, the firing rate patterns for the peak firing rates were distinct from the firing rate patterns observed in the mean firing rates across cell types. While the OFF brisk sustained RGCs had the highest mean firing rates, the ON brisk transient RGCs
had the highest peak firing rates. The ON brisk sustained cells, which had the highest mean firing rates out of all the ON types, had the lowest peak firing rates. When comparing the 3 ON and OFF types, the brisk transient cells had lower mean firing rates than the brisk sustained cells. This relationship was reversed for the peak firing rates. Additionally, the ON brisk transient cells had higher peak firing rates than their OFF counterparts while the OFF brisk transient cells had higher mean firing rates than their ON counterparts.

The differences in the mean and peak firing rate patterns across RGC types was surprising because one might expect them to be highly correlated - cells with the highest mean firing rates to have the highest peak firing rates. It is clear that these two features of the firing rate are not correlated, and are probably signaling different aspects of the visual scene for each cell type. For example, the mean spike rate might signal the overall dynamics of the stimulus, while the peak firing rate might signal more specific aspects of the stimulus. This could be useful for subsequent brain regions, like the LGN and cortex, to infer different aspects of the visual stimulus based on the readout from RGCs. For example, the LGN might learn something different about the stimulus by waiting for all the spikes from a cell and noting its mean firing rate compared to just reading out the more instantaneous firing rates (Gollisch & Meister, 2008).
5.2.2 Firing patterns across RGC types

To further examine how the neural code differed across types, the differences in firing patterns were qualitatively compared. Each type had a characteristic response pattern. For example, DS-RGCs fired sparsely and inconsistently across trials (Figure 38 a, b). ON and OFF brisk sustained RGCs fired in a sustained manner for longer periods of time, at lower mean firing rates (Figure 38 c, d, i, j). They also displayed considerable jitter in their firing rates. ON and OFF brisk and sluggish transient cells, on the other hand, fired for shorter periods of time, at high firing rates, and had lower jitter (Figure 38 e-h, k-n). OFF expanding cells fired sparsely and somewhat inconsistently across trials (Figure 38 o, p). OFF horizontal OS cells had the sparsest firing rates and the least amount of jitter in between firing epochs (Figure 38 q, r). OFF vertical OS cells fired more than OFF horizontal OS cells, with many bursts at many different instances of the WN repeats stimulus (Figure 38 s, t). The firing rate patterns were similar within cells of a type, and suggested that each RGC type processed visual information in a distinct manner.
**Figure 38**: Characteristic firing patterns and rates across RGC types. One example cell from each type is shown. Left: Spike rasters for 3 s of the WN repeats run, over 20 trials. Right: Firing rate averaged across 60 trials, plotted in the same 3 s window.
5.2.3 Predicting the firing rates of RGC types

5.2.3.1 Estimating the LN model

After inspecting the firing rates and patterns across RGC types, the LN model was used to predict the firing rates of cells to the white noise repeats stimulus. There were two parts to the LN model: a linear filter, followed by a nonlinear component. The linear filter was the spatiotemporal RF, which was estimated from the white noise stimulus using reverse correlation. Reverse correlation provided an unbiased estimate of the spatiotemporal RF (Vintch, et al., 2012), which captured how the RGC integrated the visual stimulus over space and time. The nonlinear component was a rectifying nonlinearity that transformed the signal into a firing rate. To compute the nonlinear component, the spatiotemporal RF was convolved with the white noise stimulus. The result of the convolution was a one-dimensional generator signal at each frame of the white noise stimulus. The relationship between this generator signal and the number of spikes fired at each frame was fit using an exponential function. The spatiotemporal RF and rectifying nonlinearity provided an estimate of the LN model for each cell.

5.2.3.2 Cross-validating the LN model

The LN model, which was estimated from the white noise stimulus (training data), was used to predict the response to a novel stimulus - the white noise repeats. This type of analysis (training the model with a portion of the data and testing it on new data) is called cross-validation, and provides a rigorous test of how well the model...
generalizes to new data and limits overfitting of the data. The LN model was evaluated for each cell, and was used to predict the firing rate of that cell to the white noise repeats stimulus. The predicted firing rate from the LN model was then compared to the mean firing rate from the white noise repeats stimulus. The mean firing rate was computed by averaging together 60 trials of the white noise repeats stimulus, where each trial lasted for 10 seconds.

5.2.3.3 **Qualitative evaluation of the performance of the LN model across RGC types**

Fig 39 shows the actual (black) and predicted (red) firing rates for an example cell from each type. It can be seen that the LN model does not perform consistently well for each type. It has a characteristic performance for each type, and performs better for certain types compared to other types. For example, the LN model constantly underestimated the firing rate of DS-RGCs, did not predict many of the firing events that occurred, and even predicted spikes when there were none fired (Figure 39 a). The LN model also performed poorly for the OFF expanding cells. It predicted some events but not others, and underestimated the sharp peaks (Figure 39 h). On the contrary, the LN model performed better for the ON brisk sustained RGCs, the LN model captured the mean firing rate and many of the spiking events. Nevertheless, it did not capture the troughs of the response accurately (Figure 39 b). For the ON brisk transient and sluggish transient RGCs, the LN model captured more of the spiking events (Figure 39 c, d); however it did at times predict firing events
when there were none. For the OFF brisk sustained and transient RGCs, the LN model predicted the mean firing rate, most of the firing events, and peaks and troughs of the responses well (Figure 39 e, f). The prediction from the LN model was also good for the OFF sluggish transient cells, though it did underestimate some of the peaks (Figure 39 g). In fact, the model was able to capture the structure of the spike trains of the 3 OFF types more faithfully than the 3 ON types (quantified below). For the 2 OS types, the LN model predicted the firing events and peak firing rates much better for the horizontal OS RGCs (Figure 39 i) than for the Vertical OS RGCs (Figure 39 j). The LN model thus differed in its performance on a cell type to cell type basis, and its performance for each cell type is quantified below.
Figure 39: Comparison of the actual firing rate (black) to the predicted firing rate (red) from the LN model for an example cell from each type. a. DS-RGC. b. ON BS. c. ON BT. d. ON ST. e. OFF BS. f. OFF BT. g. OFF ST. h. OFF Exp. i. HOS-RGC. j. VOS-RGC.

5.2.3.4 Quantitative evaluation of the performance of the LN model across RGC types

To quantify how well the model predicted the responses of cells to the white noise repeats stimulus, two measures were computed. The first measure was the R squared value, which determines how well the model fits the data (see Methods,
Chapter 2). The R squared value was computed for each cell and the average and standard error was plotted for each type (Figure 40a). The R-squared values confirmed that the LN model had a distinct performance for each type, and reflected the qualitative observations from the previous section. DS-RGCs, Expanding RGCs and VOS-RGCs had the lowest R-squared values, while the 3 ON, 3 OFF and HOS-RGCs had higher R-squared values. The R-squared values for the DS, Expanding and VOS cells were negative because the prediction performed worse than a straight line (the mean). One reason for this was because the predicted mean firing rates were different from the actual mean firing rates for these three cell types, and hence, the model performed worse than the mean firing rate (Figure 39a, h, j). To evaluate how the predicted and actual firing rates varied with respect to each other, without taking into account the mean firing rates, we computed a second measure: the Pearson correlation coefficient. The correlation coefficients were much higher for each RGC type compared to the R-squared values because the R-squared value is affected by the mean firing rates, while the correlation coefficient is not (Figure 40b). Although the correlation coefficient values were different, the trend in the performance of the LN model across RGC types was similar (Figure 40a, b). DS, Expanding and VOS RGCs had the lowest correlation coefficients, the 3 ON, 3 OFF and HOS types had higher correlation coefficients, and the 3 OFF types had higher correlation coefficients than the 3 ON types. This showed that
the LN model differed in its performance of the across cell types, and the pattern was consistent across different measures.

![Graph showing R-squared and correlation coefficients for each RGC type.](image)

**Figure 40:** a. Average R-squared value for each type. Each type is shaded a different color. Error bar is 1 SEM. b. Same as b, but for Average correlation coefficients.

### 5.3 Discussion

#### 5.3.1 Difference in performance of LN model across RGC types

The LN model has been widely used in the retina to encode the spiking activity of RGCs due to its simplicity, and its ability to provide a good approximation for how cells respond to white noise stimuli (McIntosh, et al., 2016). In a previous study in the retina, the LN model was shown to account for ~70% of the variance in the spiking rate of RGCs (Pillow, et al., 2005). In this study, the LN model was evaluated on individual RGCs. However, we demonstrated in this analysis that there are multiple RGC types, and each type encodes a distinct aspect of the visual scene. Moreover, cell types have unique firing patterns, and differ in terms of their mean and peak firing rates. Hence, the LN model might not generalize well across cell types, and might perform better or
worse than 70% on each cell type, depending on how each type processes visual information.

In this study we demonstrated that the LN model differed in its performance from cell type to cell type. We showed that the LN model did not explain 70% of the variance for all RGCs. Assuming that the correlation coefficient is an indirect measure of variance; it explained 60-70% of the variance for the 3 OFF types, 50-60% of the variance for the 3 ON types, and <30% of the variance for the DS, and Expanding RGCs. The LN model also performed much better at predicting the firing rates of HOS-RGCs (62%) compared to VOS-RGCs (31%). In general, the LN model performed worse at predicting the spike rates for cell types that had highly space-time dependent RFs.

There are a few possible reasons the LN model differed in its performance across RGC types. First, not all cells in the retina are well-described by a single linear and nonlinear filter. We know from previous studies that one linear filter might not capture all aspects of the visual scene that the cell responds to. For example, it has been shown that Y cells in the cat retina (Hochstein & Shapley, 1976), brisk transient cells in the guinea pig retina (Demb, et al., 2001), and parasol cells in the monkey retina (Crook, et al., 2008) are better described by a combination of multiple LN filters, or subunits. The multiple LN filters are thought to correspond to multiple bipolar cells that preprocess the stimulus independently before being pooled by the RGCs (Meister & Berry, 1999). In the same way, there might be cell types in the rat retina that are better described by
multiple linear filters (Heine & Passaglia, 2011). Further experiments and model-fitting procedures need to be carried out to determine if any of the identified RGC types fall into this category.

Second, the LN model was evaluated using a white noise stimulus and reverse correlation. Even though this provided an unbiased estimate of the linear filter (the spatiotemporal RF) (Vintch, et al., 2012), it restricted the stimulus space in which the cell’s responses were evaluated, to white noise. Not all cell types responded equally well to white noise, and some cell types were less robustly activated by white noise than other cell types. This was evident for cell types that preferred motion, like DS-RGCs, which responded more strongly to drifting gratings than white noise, and had weak spatiotemporal RFs. Expanding RGCs also responded poorly to white noise, which could explain the variability in their firing rates to the white noise repeats, and the inferior capacity of the LN model at predicting their spike rate.

Activating cells with a larger set of stimuli could allow the identification of novel stimuli that drive these RGCs more strongly than white noise. For example, certain cell types might prefer stimuli with highly skewed spatiotemporal frequencies such as natural images (Simoncelli, 2001; Koch, et al., 2006), instead of white noise, which has an even distribution of spatiotemporal frequencies. In fact, if the stimulus that a cell type prefers resembles the natural environment that these cell types operate in (Reinagel, 2001), this could provide additional insights into the ecological niche of the animal, and
the aspects of the visual scene processed by these cell types that are not captured by white noise alone. Another alternative solution to using white noise and reverse correlation techniques is to model the responses of cell types using more general methods that alleviate stimulus restrictions, such as maximizing the mutual information between stimulus and response (Sharpee, et al., 2004; Vintch, et al., 2012).

5.3.2 Limitations of the LN model

The purpose of a computational model is to provide an accurate description of how neurons transform a visual stimulus into a spike train (Pillow, et al., 2005). Though the LN model provided a good description of the spike rates of RGCs, it did not provide a complete description of how RGCs encode the visual stimulus. The model explained at most 70% of the variance in spiking for any cell type. Moreover, the model did not match the sharp peaks of the response for most cell types. The model was unable to predict the exact dynamics of the firing rate due to the following reasons.

First, the LN model does not take into account the spiking history or post-spiking dynamics of cells. It has been shown that post-spike effects such as the refractory period and bursting can impact how frequently cells fire, thereby affecting the performance of the LN model (Berry II & Meister, 1998). This is evident when looking at the characteristic firing patterns of different cell types - most cell types tend to have periods where they fire in bursts, and periods where they are very silent. Furthermore, the ISIs of RGC types (Chapter 3) also show that once a cell fires a spike, it has a very high
probability of firing another spike within a short time interval. This demonstrates the fact that firing events are not generated independently of each other, and post spiking dynamics should be included to predict the firing rates. To take into account the spiking dynamics of RGCs, a recursive LN model can be implemented. This model, also known as the Generalized Linear Model, has a post-spike feedback term, and can improve the accuracy of the firing rate predictions (Berry II & Meister, 1998; Ostojic & Brunel, 2011).

Second, the LN model assumes that each RGC is a separate unit and predicts the responses of each RGC independently. However, RGCs are not independent channels of information; they can interact with each other to produce patterns of concerted activity. For example, nearby cells of the same type tend to fire in synchrony (Berry, et al., 1997). As a result, a more precise model will have to take into consideration groups of cells rather than a single cell, and include features such as cell to cell coupling and cross-correlations across cells of a type.

Third, RGCs exhibit stochasticity in their responses to visual stimuli. They do not fire in the same exact manner, even to identical repeats of a visual stimulus (Keat, et al., 2001). Though the LN model only predicts the average firing rate and not the instantaneous spike times of RGCs, this can impact the performance of the LN model, especially when there is trial to trial variability in firing. A more precise model that is able to predict the spike times of RGCs while taking into account their variability is needed, to better predict the firing rates of RGC types.
All in all, the LN model provides a good approximation to the neural code. It differs in its performance across cell types, providing further evidence that each type processes a distinct aspect of the visual scene. Future work should continue to probe RGCs with novel stimuli and better computational models, to completely understand the neural code and what the retina communicates to the brain.
6. Conclusions

In this thesis, we demonstrated the functional diversity of RGC types in the rat. To characterize their functional diversity, we recorded from hundreds of RGCs using a large-scale multi-electrode array and diverse visual stimuli. In Chapter 3, we developed a novel classification approach to group RGCs into distinct functional types. This approach was quantitative, verified cell types by checking for the presence of RF tiling, and reproducibly identified 13 RGC types across multiple preparations. The types consisted of 4 DS, 3 ON, 3 OFF, 1 Expanding and 2 OS types. Each type had distinct spatiotemporal RFs, ISIs, and characteristic responses to the drifting gratings, full field pulses and white noise repeats stimuli, establishing that each type carried out a distinct function.

This classification approach improved our understanding of visual processing in the following ways. First, it informed us about the aspects of the visual scene extracted at the level of the retina. It showed that there were 3 distinct ON and OFF channels processing visual information at different spatial scales, temporal scales and spike rates. It also revealed 3 new aspects of visual information that were computed in the rat retina: stimulus expansion, horizontal orientations and vertical orientations.

Having a better understanding of the aspects of the visual scene extracted in the retina can lead to some potential follow-up questions to be investigated in future work. One example would be how downstream visual areas like the cortex integrate input
from horizontal and vertical OS RGCs to give rise to orientation selectivity. Another question would be to trace where Expanding RGC axons project to, and understand how that influences locomotor behavior, such as escaping from predators. This will give us a better understanding of how the visual information processed by the retina is integrated in higher visual areas to give rise to behavior, and our perception of the world.

In Chapter 4, we carried out an in-depth quantification of the spatiotemporal properties of the distinct RGC types. We found that the ON and OFF brisk sustained, brisk transient and sluggish transient types partitioned the encoding of spatiotemporal stimuli in a manner distinct from cats and primates (Troy & Robson, 1992; Chichilnisky & Kalmar, 2002): RGCs with larger spatial integration exhibited more low-pass temporal integration, and pairs of ON and OFF RGCs exhibited contrasting functional asymmetries in their spatial and temporal RF structure. We also found that RGC types exhibited RFs that were not independent in space and time. These departures from independence arose from several distinct causes across types, and collectively indicated that the linear integration of visual signals was incompletely captured by a single pair of spatial and temporal filters. Additionally, we showed that the two OS types were functionally asymmetric. Specifically, HOS-RGCs had smaller spatial RFs, prolonged temporal integrations and a linear RF that predicted their orientation tuning.

These results improved our understanding of visual processing in the following three ways. First, it indicated that the rodent retina had a distinctive functional
organization from other mammalian species. Unlike RF tiling, which has been demonstrated across various species, the spatiotemporal integration of signals and symmetry of ON and OFF pathways differed across species. Hence, they were not general principles of organization in the retina. These differences in retinal organization may reflect differences in the organization of visual processing in downstream areas such as the primary visual cortex (Laramee & Boire, 2015). They could also reflect differences in the ecological niche of the animal: rodents and primates move about in different environments, and different environments can exhibit different spatial patterns (Oliva, et al., 1999; Torralba & Oliva, 2003). Additionally, they might reflect the physical constraints imposed by the small and lateralized eyes in rodents, which may have produced differences in retinal organization compared to species with larger eyes and more binocular vision (Collins, 1999; Huberman & Niell, 2011). Interesting future work here could be to compare and contrast the organizational principles of ON and OFF types across species. For example, the retinal organization in the rat could be compared to the tree shrew, which is starkly different from the rat: it has a cone dominated retina and is a highly visual animal (Muller & Peichl, 1989). This can provide additional insight into how the principles of retinal organization are influenced by the features of the animal and its role in the environment.

Second, the SVD analysis demonstrated that the retina carried out a diverse set of complex computations. It showed that the retina was not just a simple spatio-temporal
filter (Meister & Berry, 1999), and that the spatial and temporal components of the RFs of RGCs interacted in complex ways when processing visual information. Comprehending the complexity with which RGCs represent spatial and temporal information can enable us to better understand the signals and information conveyed to downstream visual areas. From this, we can gain a better appreciation for how these signals are integrated together by cells in downstream visual areas to refine the visual image and give rise to visual perception and behavior.

Third, the finding that horizontal and vertical orientations were encoded at different spatiotemporal scales with different degrees of space-time dependence showed that horizontal and vertical OS were encoded using different mechanisms. This could lead to follow up work that examines how differences in presynaptic circuitry give rise to 2 different types of OS (Venkataramani & Taylor, 2010). The asymmetric encoding of horizontal and vertical orientations could also indicate how these orientations are represented in the visual world, and provide some clues as to how the retina might have evolved to accommodate these orientations. For example, in natural scenes, vertical orientations are less prevalent than horizontal orientations (Hansen & Essock, 2004). To account for the fact that vertical orientations are observed less frequently, it could be possible that the retina evolved to encode vertical orientations in a more complex manner. A more complex encoding of vertical orientations might allow the retina to
capture additional visual features about vertical orientations, and generate a salient representation of them in higher visual areas.

In Chapter 6, we compared the firing patterns of distinct RGC types, and quantified the performance of the LN model at predicting their responses. We found that each type had a distinct firing pattern, and that the LN model differed in its performance across types. Evaluating the performance of the LN model across cell types was useful because it identified RGC types where white noise analysis and reverse correlation failed to capture the extent of how the cell was spatially and temporally integrating visual information. This can motivate future work to develop better computational models and use diverse visual stimuli to completely characterize the neural code of the retina. A complete understanding of the neural code will mean that we have fully understood the computation carried out by RGCs, and that we can predict its response to any new visual stimulus, which could aid in the development of better prosthetic devices.

All in all, this thesis, through the use of multi-electrode arrays and diverse visual stimuli, a novel classification approach, and computational analysis and modeling techniques demonstrated that the computations performed in the rat retina were incredibly diverse and complex. Future work should continue to understand cell type diversity and computations in the retina, which will further our knowledge about how
the retina processes visual information, what it conveys to the brain, and how this contributes to our percept of the visual world.
References


Anishchenko, A. et al., 2010. Receptive field mosaics of retinal ganglion cells are established without visual experience. *Journal of Neurophysiology*, 1 April, 103(4), pp. 1856-1864.


Gauthier, J. L. et al., 2009a. Receptive fields in primate retina are coordinated to sample visual space more uniformly. PLoS Biology, 7 April, 7(4), p. e1000063.


Jepson, L. H. et al., 2013. Focal electrical stimulation of major ganglion cell types in the primate retina for the design of visual protheses. *Journal of Neuroscience, April, 33*(17), pp. 7194-7205.


Sun, W., Li, N. & He, S., 2002b. Large-scale morphological survey of mouse retinal ganglion cells. *Journal of Comparative Neurology*, 16 September, 451(2), pp. 115-126.


Biography

Sneha Ravi was born on December 15, 1987 in India. She received her Bachelor’s degree in Bioengineering from the University of Michigan (Ann Arbor) in 2009, and her Master’s degree in Bioengineering from the University of Pennsylvania in 2011. Her Master’s Thesis was completed under the guidance of Dr. Robert Smith and titled “Horizontal Cell Feedback is Mediated by Ephaptic Interactions in the Retina”. In 2011, she matriculated into the Bioengineering PhD program at University of Southern California (USC), and in 2015, she transferred to the Duke Neurobiology PhD program. She is completing her PhD at Duke under the guidance of Dr. Gregory Field. During her PhD, she received the Viterbi School of Engineering Doctoral Fellowship (USC, 2011-2014), the Joseph and Eula Lawrence Travel Scholarship (ARVO, 2013), and the Grodins Symposium Service Award (USC, 2013). She has held professional affiliations in the following societies, and presented her work at their meetings: Society for Neuroscience (2015) and Association for Research in Vision and Ophthalmology (2013). After completing her PhD, she will be attending the Insight Data Science Post-Doctoral Fellowship in 2017 to continue her career in computational data analysis and modeling.