Contributions of Specific Retinal Circuits and Their Respective Projections to Visual Behaviors

Jenna Mackenzie Krizan
Washington University in St. Louis

Follow this and additional works at: https://openscholarship.wustl.edu/art_sci_etds

Part of the Neurosciences Commons

Recommended Citation

This Dissertation is brought to you for free and open access by the Arts & Sciences at Washington University Open Scholarship. It has been accepted for inclusion in Arts & Sciences Electronic Theses and Dissertations by an authorized administrator of Washington University Open Scholarship. For more information, please contact digital@wumail.wustl.edu.
Contributions of Specific Retinal Circuits and Their Respective Projections to Visual Behaviors

by

Jenna Krizan

A dissertation presented to
The Graduate School
of Washington University in
partial fulfillment of the
requirements for the degree
of Doctor of Philosophy

August 2022
St. Louis, Missouri
# Table of Contents

List of Figures

Acknowledgments

Abstract

Chapter 1: Introduction

1.1 Visual Processing in the Mouse Retina

1.2 Direction selectivity in the retina and decussating pathway to the brain

1.3 Ipsilaterally projecting RGCs and their contributions to binocular vision

1.4 Superior colliculus: A hub for integrating diverse visual inputs and motor outputs

1.5 Scope of the dissertation

1.6 References

Chapter 2: Direction Selectivity and Predation in Mice

2.1 Introduction

2.2 Method

2.3 Results

2.4 Discussion

2.5 References

Chapter 3: Contributions of Ipsilaterally Projecting Sustained ON and OFF Alpha Retinal Ganglion Cells to Predation

3.1 Introduction

3.2 Method

3.3 Results

3.4 Discussion

3.5 References

Chapter 4: Conclusions and Future Directions

4.1 Conclusions

4.2 Future Directions

4.3 References
List of Figures

Figure 1.1: Visual processing in the mammalian retina.................................................15
Figure 1.2: Outputs of DSGCs to the brain.................................................................19
Figure 1.3: Ipsilateral and contralateral RGC innervation to SC...............................23
Figure 2.1: Chemogenetic silencing of NF cells in sSC impairs predation...................50
Figure 2.2: CNO administration does not induce predatory deficits in the absence of iDREADDs expression.................................................................51
Figure 2.3: NF cell ablation phenocopies pharmacogenetic silencing.......................53
Figure 2.4: Predation persists following chemogenetic silencing of V1......................55
Figure 2.5: AOS nuclei make minimal contributions to predation.............................56
Figure 2.6: Discrete visual pathways drive distinct visual behaviors.......................58
Figure 2.7: NF cells in sSC are innervated by ooDSGCs........................................60
Figure 2.8: Intraocular injection of DT ablates SACs and abolishes retinal DS........61
Figure 2.9: Predation persists following the loss of retinal DS prior to training........62
Figure 2.10: DT-induced ablation of SACs follows a stereotyped time course..........65
Figure 2.11: Loss of retinal DS that coincide with test does not prevent efficient hunting......67
Figure 3.1: Genetic intersection of SERT-Flp and KCNG4-Cre labels a subpopulation of RGCs in ventrotemporal retina.................................................................82
Figure 3.2: SERT-KCNG4 RGCs project ipsilaterally.............................................83
Figure 3.3: SERT-KCNG4 RGCs are αRGCs......................................................84
Figure 3.4: Electrophysiological profiling identifies SERT-KCNG4 RGCs as sONα and sOFFα RGCs.................................................................85
Figure 3.5: Ipsi-sONα and ipsi-sOFFα RGCs form independent translaminar mosaics........86
Figure 3.6: Ipsi-αRGC projections to subcortical visual centers.............................87
Figure 3.7: Tox expression cleaves VAMP2 from ipsi-αRGC terminals. ......................................89

Figure 3.8: Ipsi-αRGCs guide efficient predation.................................................................90

Figure 3.9: Behavioral phenotypes are recapitulated between three visual system manipulations. .................................................................................................................................92

Figure 3.10: Ipsi-sONα RGCs directly innervate NF cells in sSC. ...............................93
Acknowledgments

I consider myself lucky to be surrounded by people – family, friends, coworkers, and mentors – that have been supportive of my goals, while also reminding me that balance is important. My graduate school career has been 6 years of personal and professional growth, and for that, I am grateful.

Thank you to the members of my thesis committee: Drs. Yao Chen, Tim Holy, Peter Lukasiewicz, and Steve Mennerick. You have been an invaluable sounding board scientifically, personally, and professionally. I appreciate all the time and effort that you all put into mentoring me – even as the scope of my thesis swerved out of everyone’s areas of expertise. Every interaction has been insightful and a joy. I hope to continue drawing on your experiences as I move forward with my career.

To Tim: Thank you for recruiting me to WashU. Your kindness and enthusiasm were infectious during recruitment and showed me the kind of welcoming learning environment that this University could foster. Knowing that I had a rotation in your lab to look forward to helped buffer the uncertainty of moving to the Midwest. I am so glad that I took the risk. I can’t imagine a better place to have received my graduate training and I doubt that I would have immersed myself so deeply in the community at another institution. I feel privileged to have had your mentorship in a variety of capacities for the last several years.

To Daniel: Thank you for cultivating a lab that often felt more like a playground than a work environment. When I decided that I wanted to study sensory systems, I knew that learning the techniques to do so would be challenging. I expected and was prepared to spend several years mastering a single technique to answer a single, unspecified question. Instead, every day in the
lab greeted me with a new challenge to solve or project to work on. Thank you for pushing me to learn as much as I have, for always being available to help when needed, and for encouraging me to explore my interests in and out of the lab.

Together, the five of you formed the bedrock of my graduate education and laid a foundation that I am confident to build upon. That is invaluable.

Thank you to my lab mates and friends – old and new – for inspiring me daily, pulling me out of my comfort zone, and keeping me grounded.

My family gifted me curiosity and instilled my work ethic. For 28 years, they have encouraged me to seek out and seize opportunities that excite me. They have supported every goal that I have set for myself – including, but not limited to, the decision I made when I was 16 years old to get a PhD in Neuroscience. I am grateful beyond words to achieve this goal and for them to revel in my success with me.

Finally, the last 6 years of my education would not have been possible without generous funding from the National Institute of Health to Daniel and the Department of Ophthalmology & Visual Sciences.

Jenna Krizan

Washington University in St. Louis

August 2022
For my family.
ABSTRACT OF THE DISSERTATION

Contributions of Specific Retinal Circuits and Their Respective Projections to Visual Behaviors

by

Jenna Krizan

Doctor of Philosophy in Biology and Biomedical Sciences

Neurosciences

Washington University in St. Louis, 2022

Professor Daniel Kerschensteiner, Chair

The survival of a species is inextricably linked to its ability to successfully navigate and interact with its surroundings, whether to seek safety from predators or gain sustenance from prey. Both functions are performed by mice, guided by vision, and rely on intricate processing in the retina and subcortical targets in the brain. This dissertation addresses how specific features of the visual environment and specific retinal ganglion cell circuits that sample a particular space in the visual environment are used to guide efficient predation in mice.

Recent studies have begun to link the ability to detect, track, and ultimately capture prey to the retina and its projections to the superficial layers of superior colliculus (sSC). More than 85% of retinal ganglion cells (RGCs) that encode individual features of the visual environment project to SC, resulting in SC neurons that integrate and encode multiple visual features. How a single feature of visual space is utilized to guide complex behaviors and how individual retinal ganglion cells contribute to these computations remain unknown.

Here, we show that narrow-field (NF) cells in sSC, which encode object size, speed, and motion direction, are required for accurate approaches toward prey. We show that other visual pathways that encode motion direction are not required for predation and test specifically whether NF cells...
must encode this feature for mice to accurately target prey. We manipulate the presynaptic retinal circuitry to remove the directional tuning of NF cells, while maintaining retinal excitatory drive through sSC. We show that mice remain able to accurately approach, pursue, and capture prey, despite being unable to extract information about how it moves. We then sought to determine which of the RGC types responsible for binocular vision guide predation. Leveraging an intersectional genetic strategy, we silence a subpopulation of 2 ipsilaterally projecting RGCs that can signal prey (ipsi-αRGCs, ~260 total cells) and find that predation is disrupted, consistent with the deficit caused by silencing the NF cells to which they project. Together, these results suggest 3 main findings. First, visual behaviors are guided by specific visual pathways and sSC is required for predation. Second, information about motion direction is not required for efficient predation, despite it being a key feature of prey. This suggests that the ability of cells in sSC to integrate and signal multiple types of visual information is more important than the ability to reliably encode a single feature of interest. Third, 2 subtypes of ipsi-αRGCs can account for the role of binocular vision in predation and might indicate that reliable encoding of a sliver of visual space is most important for efficient predation.
Chapter 1: Introduction

The survival of a species is inextricably linked to its ability to successfully navigate and interact with its surroundings, evaluate the safety of its environment, and forage or pursue and capture prey. Each of these functions – successful navigation (Sabbah et al. 2017; Yoshida et al. 2001), evaluation of safety (T. Kim et al. 2020; Yilmaz and Meister 2013), and pursuit of prey (Hoy et al. 2016; Johnson et al. 2021) – is guided by vision and is dependent on extensive processing that occurs in the retina to extract relevant features of the visual scene. Despite a growing understanding of the computations that are performed by the output neurons of the retina, how information about features of visual space is used by downstream pathways in the brain to inform meaningful behaviors remains elusive (Dhande et al. 2015). This dissertation aims to understand how specific populations of retinal output neurons and their respective projections contribute to complex visual behaviors such as predation, by 1) encoding motion direction or 2) supporting binocular vision.

This chapter is divided into 4 parts that, together, frame the current state of our thinking about how the visual system – from the level of individual retinal circuits or subpopulations of cells to downstream visual pathways in the brain – is used to guide a mouse through the complex stages of detecting, pursuing, and capturing prey. I first introduce visual processing in the retina in terms of the basic neural architecture required to convert photons into meaningful electrical signals. I then discuss the circuit components required to compute motion direction in the retina and how this information is sent via a conventional decussating pathway to visual targets in the brain. I contrast this with a unique population of retinal ganglion cells that remain uncrossed at
the optic chiasm to inform a minority of subcortical nuclei about stimuli in the binocular visual field. Finally, I discuss in depth the overlap between these pathways (i.e., direction-selective and ipsilaterally projecting) and explore the cell types and processing capabilities of the superficial superior colliculus.

1.1 Visual processing in the mouse retina

All information about visual space must be detected, processed, and routed through the retina before it can be integrated downstream to inform behavioral outputs. In support of this, the retina has a laminar structure composed of alternating nuclear and synaptic layers that aids in transforming pixel-like information about light intensity to information about salient features of visual space (see Figure 1.1). Five broad classes of neurons in the retina support this transformation and are introduced below.

![Visual processing in the mammalian retina](image)

Figure 1.1. Visual processing in the mammalian retina. Photoreceptors (PRs) in the outer nuclear layer (ONL) of the retina convert light to electrical signals and relay them to horizontal cells (HC) and bipolar cells (BC) in the...
outer plexiform layer (OPL). BCs then relay this information from the outer to the inner retina and form complex synapses with amacrine cells (AC) and retinal ganglion cells (RGCs) in the inner plexiform layer (IPL). ACs modulate the outputs of BCs to generate unique visual tuning properties of the RGCs that project visual information to the brain.

Light first enters the eye, traverses the transparent cells of the retina, and is focused onto the outer segments of the photoreceptors (PRs). Three types of PRs (rods and 2 cone subtypes) vary in their absolute and spectral sensitivities and are distributed differentially across the mouse retina (Masland 2001; Wässle 2004). Together, the 3 PR types transduce photons to glutamatergic signaling that encodes pixel-like information about increments and decrements in absolute light intensity.

Glutamatergic signaling from PRs is modulated by a single horizontal cell type (Masland 2001; Wässle 2004) and feeds forward as it is transmitted to 15 types of bipolar cells (BCs) in the inner retina (Shekhar et al. 2016; Franke et al. 2017; Della Santina et al. 2016). BCs then use glutamate, in parallel, to encode information about contrast (ON vs. OFF) and kinetics (transient vs. sustained). Depending on their respective response properties, BC terminals ramify at varying depths of the inner plexiform layer (IPL), where BC terminals selectively converge and synapse with postsynaptic amacrine cells (ACs) and retinal ganglion cells (RGCs) (Euler et al. 2014).

The spike trains of the 40+ types of RGCs encode specific features of the visual environment and provide the sole information output from the retina to the brain (Tran et al. 2019; Baden et al. 2016; Bae et al. 2018). The feature-selective responses of RGCs arise due to the lateral interactions between BCs, RGCs, and 60+ types of predominantly inhibitory ACs (Yan et al. 2020; Franke and Baden 2017). In many cases, the same BCs that drive the responses of downstream RGCs also drive the responses of ACs that can modulate, shunt, amplify, or complement the glutamatergic release from BCs onto RGCs, thus resulting in net-tuned outputs at the level of RGC spike trains. In turn, these RGCs converge to innervate at least 59 subcortical
visual targets in the brain in a cell-type specific manner to guide downstream image-and non-image-forming visual processes (Martersteck et al. 2017; Morin and Studholme 2014).

1.2 Direction selectivity in the retina and decussating pathway to the brain

Computation of direction selectivity in the retina

Compared to the retinas of other species, the mouse retina is rich in RGCs that detect specific features of the visual environment. Approximately 15% of RGCs encode motion direction in their spike trains by firing maximally to a single direction of motion that is oriented along the cardinal or body axes (Kay et al. 2011; Barlow and Hill 1963). This pattern of responses arises from the integration of feedforward excitatory inputs from BCs and directionally tuned inhibition from starburst amacrine cells (SACs).

In the mouse retina, motion direction is first detected in the neurites of SACs (Euler, Detwiler, and Denk 2002). SACs constitute a population of non-spiking, dual-transmitter interneurons that stratify in either the ON or OFF layers of the IPL (O’Malley, Sandell, and Masland 1992; Lee, Kim, and Zhou 2010; Famiglietti 1983). SACs have radially symmetric neurite arbors that integrate excitatory and inhibitory inputs near the soma and release GABA and acetylcholine in the arbor periphery (Vlasits et al. 2016; Ding et al. 2016; Park et al. 2014; Greene et al. 2016; Famiglietti 1991; Fransen and Borghuis 2017). The biophysical properties of and distribution of inputs along their arbors permit SAC neurites to independently signal movement outward from the soma (i.e., centrifugal motion) via the graded release of GABA and acetylcholine (Euler, Detwiler, and Denk 2002; Hausselt et al. 2007; Koren, Grove, and Wei 2017).
SACs tile extensively across the retina and selectively form synapses with their postsynaptic partners, such that individual postsynaptic RGCs receive inputs from SACs that signal the same direction of motion (Briggman, Helmstaedter, and Denk 2011; Yonehara et al. 2011; Wei et al. 2011; Fried, Münch, and Werblin 2002). The resulting direction selective RGCs (DSGCs) are therefore suppressed by SACs in response to the same direction of motion (i.e., null direction), permitting them to fire maximally in response to the opposite direction of motion (i.e., preferred direction) (Barlow and Hill 1963). SAC-DSGC inhibition is the primary mechanism by which DSGC spiking becomes directionally tuned; however, few instances of asymmetric excitation from BCs and cholinergic inputs from SACs have been reported that might complement asymmetric inhibition under various stimulus conditions (Fried, Münch, and Werblin 2002; Sethuramanujam et al. 2021; Sun et al. 2006; Matsumoto, Briggman, and Yonehara 2019).

DSGCs can be divided into 2 predominant populations (see Figure 1.2): 1) those responding exclusively to bright stimuli (i.e., ON-DSGCs or oDSGCs) and 2) those responding to both bright and dark stimuli (i.e., ON/OFF-DSGCs or ooDSGCs). oDSGCs and ooDSGCs share a common mechanism by which they generate direction-selective (DS) responses (i.e., asymmetric inhibition from SACs) and both provide decussating streams of visual information to the brain. Yet, the image features to which oDSGCs and ooDSGCs respond, the axes to which their responses are aligned, and the brain regions to which they project differ, suggesting different roles in guiding visual behaviors.
Figure 1.2. Outputs of DSGCs to the brain. SACs use GABA to preferentially tune the responses of DSGCs, such that they respond optimally to a single direction of motion and are silenced in response to the opposite direction of motion. oDSGCs (blue) respond to light increments and project primarily to the nuclei of the accessory optic system, which is parcellated by the preferred motion direction of its inputs. oDSGCs that prefer vertical motion project to the medial terminal nucleus (MTN), whereas oDSGCs preferring horizontal motion project to the dorsal terminal nucleus (DTN) or nucleus of the optic tract (NOT). In contrast, ooDSGCs (orange) respond to both light increments and decrements and project to the dorsolateral geniculate nucleus (dLGN) and superficial colliculus (SC).

Direction selective pathways in the brain

oDSGCs respond to bright stimuli and signal motion that is aligned to the body axes (i.e., upward, downward, and forward), responding optimally to large, slow-moving stimuli (Dhande et al. 2013; Sabbah et al. 2017; W. Sun et al. 2006). The axons of oDSGCs cross at the optic chiasm and selectively innervate the nuclei of the accessory system (AOS). oDSGCs preferring vertical motion preferentially innervate the medial terminal nucleus (MTN), whereas oDSGCs preferring horizontal motion instead innervate the dorsal terminal nucleus (DTN) and nucleus of the optic tract (NOT) (Yonehara et al. 2009; Dhande et al. 2013; Yonehara et al. 2008; Osterhout et al. 2015; Martersteck et al. 2017). The nuclei of the AOS are dominated by inputs from oDSGCs. Except for a type of ooDSGCs that encodes slow, horizontal motion, inputs from ooDSGCs are excluded from the AOS (Dhande et al. 2013). The large size and slow motion preferences of oDSGCs suit them to respond to global motion indicative of slow head and body movements (i.e., self-motion) (Sabbah et al. 2017; Dhande et al. 2013). These inputs to AOS are
evolutionarily conserved across species and used to generate gaze-stabilizing eye movements (i.e., the optokinetic reflex) that compensate for self-motion (Simpson 1984; Yoshida et al. 2001; Cahill and Nathans 2008).

In contrast, ooDSGCs respond to both bright and dark stimuli and signal the motion of small objects moving quickly along the 4 cardinal directions (i.e., dorsal, ventral, temporal, and nasal) (Barlow, Hill, and Levick 1964; Yonehara et al. 2013; Rivlin-Etzion et al. 2011; Kay et al. 2011; Huberman et al. 2009). Like oDSGCs, ooDSGCs cross at the optic chiasm, however, they instead innervate the shell region of the dorsolateral geniculate nucleus (dLGN) and superficial layer of the superior colliculus (sSC) (Huberman et al. 2009; Martersteck et al. 2017; Ellis et al. 2016). In both dLGN and sSC, ooDSGC inputs are integrated with heterogeneous inputs from other RGC types (Reinhard et al. 2019; Jiang et al. 2022) to contribute 1) to the directional tuning of and 2) excitatory drive through these nuclei.

To date, the function of direction selectivity is best understood at the microcircuit level and considerable progress has been made in disentangling the synaptic, dendritic, cellular, and circuit components that enable motion direction to be computed across many stimulus conditions. While DS pathways have been mapped through the brain and oDSGC-mediated signaling through the AOS is required for the optokinetic reflex, how ooDSGC-mediated signaling through dLGN and sSC contribute to visual behavior has remained elusive.

Previous studies suggested that thalamocortical cells in the dLGN shell preferentially received inputs from ooDSGCs (Cruz-Martín et al. 2014; Martersteck et al. 2017). More recent evidence demonstrates convergence of ooDSGCs and other RGC types, resulting in thalamocortical cells that range widely in their degree of directional tuning (Jiang et al. 2022). Moreover, the complex
integration of non-DS inputs (i.e., ooDSGCs rendered non-DS by ablation of SACs and other non-ooDSGC RGCs) to the dLGN-primary visual cortex (V1) pathway is sufficient to compute motion direction de novo in V1 (Hillier et al. 2017). This pattern of results suggests that the contribution of ooDSGCs to the dLGN-V1 pathway ranges beyond their DS tuning. In contrast, the tuning of retinocollicular neurons in sSC depends on the DS tuning of retinal input, despite local SC circuits receiving feedback from higher visual areas, such as V1 (Shi et al. 2017; Ahmadlou, Tafreshiha, and Heimel 2017). Thus, these results suggest that the ability to rapidly encode motion direction in sSC might be more central to informing visual behaviors mediated by sSC than those mediated by the dLGN-V1 pathway.

Elements of the visual scene that move are often important for an animal to attend to, as they can indicate the approach of a predator or availability of prey. Thus, an animal’s ability to reliably detect and encode the movement of such a stimulus is beneficial to survival. Many pieces of the visual circuits that enable a mouse to engage in defensive behaviors (i.e., in response to a predator) have been elucidated (T. Kim et al. 2020; Yilmaz and Meister 2013). In contrast, what visual information is used to guide appetitive hunting behaviors is largely unknown. Given the ability of mice to hunt insects and the small size, dark contrast and rapid movement trajectories of prey such as crickets (Hoy et al. 2016; Hoy, Bishop, and Niell 2019), we reasoned that ooDSGCs might encode stimulus features useful to engage in predatory behaviors mediated by sSC. We test for a role of direction selectivity in predation in Chapter 2 of this dissertation.
1.3 Ipsilaterally projecting RGCs and their contributions to binocular vision

In the mouse, most RGCs, like DSGCs, send their axons from the retina to the brain such that their axons cross at the optic chiasm to inform contralateral brain areas (see Figure 1.3). As a result, RGCs that cover the entire retina innervate 34 visual targets to sample both central and peripheral visual space (Martersteck et al. 2017; Morin and Studholme 2014). In contrast, a small percentage of RGCs (~2%) remain uncrossed at the optic chiasm to instead innervate small, ipsilateral regions of a subset of retinorecipient targets. The RGCs that send their axons ipsilaterally (ipsi-RGCs) constitute a small population of RGCs in the ventrotemporal sliver of retina that encodes visual space dorsal to the mouse’s nose, or immediately in front of the mouse when its head is pitched forward (Johnson et al. 2021). As a result, the ipsi-RGCs from each eye converge to encode an overlapping region (~40°) of central visual space (i.e., the binocular visual field) (Johnson et al. 2021; Michaiel, Abe, and Niell 2020; Hoy et al. 2016).
Figure 1.3. Ipsilateral and contralateral RGC innervation to SC. At least 85-90% of RGCs project to SC and most RGC axons cross the midline at the optic chiasm to innervate the superficial layers of SC. Contralaterally projecting RGCs (grey, blue) can originate anywhere in the mouse retina and completely sample visual space. The depth at which their terminals ramify in SC depends on their cell type identity. For example, ooDSGCs (blue) broadly innervate the upper layers of sSC and other contrast-encoding or melanopsin-expressing RGCs innervate the lower layers of superficial SC. In contrast, ipsi-RGCs (yellow) originate only from a region of temporal retina, do not cross the midline at the optic chiasm, and project sparsely to the lower layer of sSC.

The size of the binocular visual field depends on the lateralization of eye orbits, such that predators with front-facing eyes have the largest binocular visual fields and prey with lateral eyes have the smallest area of overlap (Heesy 2008). In species with highly developed visual systems, such as primates, nearly 50% of RGCs project ipsilaterally (Stone, Leicester, and Sherman 1973), resulting in a binocular visual field that is richly innervated by RGCs that encode the full complement of visual features computed by the retina. In contrast, predatory species with less developed visual systems, such as the cat, can support a large binocular visual field but have more mixing of ipsilaterally and contralaterally projecting RGCs in the temporal retina (Stone and Fukuda 1974). However, this mixing is non-random and binocular vision is preferentially supported by contrast-encoding RGCs (Stone and Fukuda 1974). Mice have more lateral eyes
and a less developed visual system than either primate or cat, but their smaller binocular visual field encompasses the same organizational scheme: a small subset of ipsi-RGCs in temporal retina, many of which encode contrast (Johnson et al. 2021).

While less developed than that of more specialized hunters, the binocular visual field of mice can confer the same visual advantages that have evolutionarily favored predators. The integration of visual information between eyes can improve stereoscopic depth perception, enable the detection of camouflaged prey, and – key to nocturnal hunters – improve visual acuity under dim light conditions (Heesy 2008; Pettigrew 1986). Individually or in combination, these theoretical advantages of a functional binocular visual field all improve the ability of a predator to detect, pursue and capture its prey.

Mice predate insects and pitch their heads forward during pursuit to maintain their prey in the center of their binocular visual field (Johnson et al. 2021). The ability to maintain accurate bearing toward prey is dependent on ipsi-RGCs, suggesting a central role of binocular vision in predation. In mice, only 9 of ~40 types of RGCs project ipsilaterally and these include select RGCs that 1) encode contrast, 2) express melanopsin and are intrinsically photosensitive, or 3) are suppressed by contrast (Johnson et al. 2021). How these types of information are used in the binocular visual field and whether all contribute to predation remains unknown. However, the apparent reliance of the binocular visual field of the cat on contrast-encoding RGCs and the presence of contrast-encoding RGCs in the binocular visual field of mice together suggest an evolutionarily conserved need for binocular contrast encoding (Stone and Fukuda 1974). Consistent with this, of the 9 ipsi-RGC types in mice, only the contrast-encoding and suppressed-by-contrast RGCs (5 RGC types, total) can reliably encode stimulus features that are consistent with moving prey (Johnson et al. 2021). Developing a functional understanding of how binocular
vision is used to guide predation in the mouse therefore can offer understanding into the functional organization of the visual system, as shared between mammalian predators. To this end, a role of contrast encoding within the binocular visual field in predation is tested in Chapter 3 of this dissertation and the downstream pathways by which this information might be used to execute behaviors are considered.

1.4 Superior colliculus: A hub for integrating diverse visual inputs and motor outputs

Together, contralaterally and ipsilaterally projecting RGCs innervate 59 subcortical targets in the brain (Martersteck et al. 2017). However, the ooDSGCs and subset of contrast-encoding ipsi-RGCs that are central to this dissertation converge in the superficial layers of superior colliculus (sSC) (Reinhard et al. 2019). In mice, neural circuits in SC are necessary and sufficient for hunting. Genetically defined classes of neurons in sSC are required for detection and pursuit of prey (Hoy, Bishop, and Niell 2019), whereas the activation of circuits projecting out of SC are sufficient to induce predatory behaviors (Zhao et al. 2019; Shang et al. 2019). Across species, SC has additional roles in directing attention, shifting gaze, and orienting toward salient features in the visual environment (Wurtz and Albano 1980; Stubblefield, Costabile, and Felsen 2013; Sahibzada, Dean, and Redgrave 1986). We therefore consider sSC as a hub that can integrate diverse visual inputs – including those from DSGCs and ipsi-RGCs – to generate the motor commands necessary to predate.

Organization of SC
SC integrates visual inputs from the retina and V1 with other sensory modalities to identify and direct attention to salient features of the environment and translate sensory input to motor outputs. Despite the diversity of information that SC integrates, its inputs are highly ordered and correspond to the laminar structure of SC. Whereas deep SC receives multimodal inputs and performs pre-motor computations, the superficial layers are dominated by visual inputs (Cang et al. 2018; Benavidez et al. 2021; Wurtz and Albano 1980). Visual inputs are further organized by visual space in a 2D retinotopic map, which is preserved across SC depth, as all visual and multimodal inputs and motor outputs are aligned to this mapping (Cang and Feldheim 2013; Seabrook et al. 2017).

In addition to the retinotopic map that is established along the rostro-caudal and medio-lateral axes, visual inputs to sSC vary by cell type and depth. The most superficial layers of sSC receive contralateral input from ooDSGCs, whereas the lower layers of sSC receive inputs from contralaterally projecting aRGCs and V1 and clusters of ipsi-RGCs (Ito and Feldheim 2018). As SC has increasingly been adopted as a model visual system, there have been efforts to uncover microcircuits in sSC that extend beyond this laminar organization (i.e., columns) that represent the precise organization of visual inputs by function in addition to retinotopy. Despite some mixed reports of clusters of similarly tuned DS inputs to sSC (de Malmazet, Kühn, and Farrow 2018), systematically there is no evidence for this level of organization in the mouse (Chen et al. 2021). Instead, individual retinocollicular neurons in sSC appear to receive heterogeneous inputs from ~6 RGCs that primarily encode similar areas of visual space (Reinhard et al. 2019; Chandrasekaran, Shah, and Crair 2007). The integration of these heterogeneous inputs in sSC then results in retinocollicular neurons that are tuned with novel response properties.

*Cell-type specific contributions of sSC to predation*
Four cell types predominate sSC that vary in their morphological and physiological profiles (Gale and Murphy 2014). Of these 4 cell types, wide-field (WF) cells are required for the detection of prey and narrow-field (NF) cells are engaged during the pursuit and capture of prey (Hoy, Bishop, and Niell 2019). Both WF and NF cells have somas in lower sSC that extend dendrites covering the vertical span of sSC to therefore sample diverse retinal inputs (Gale and Murphy 2014; Cang et al. 2018). As a result of their sampling strategies, WF and NF cells respond optimally to different sets of visual stimulus features that are consistent with their respective roles in guiding predation.

WF cells extend large, branched dendrites obliquely to the surface of sSC, permitting them to sample RGC inputs that cover up to 1000 deg² of visual space (Gale and Murphy 2014; Hoy, Bishop, and Niell 2019). Despite their large receptive field, the responses of WF cells are constrained by local inhibition from horizontal cells (Gale and Murphy 2016). A combination of lateral inhibition and putative input from object motion-sensitive and contrast-encoding RGCs therefore primes WF cells to trigger dendritic spikes in response to small objects that move slowly anywhere within their receptive field (Gale and Murphy 2016, 2014; Hoy, Bishop, and Niell 2019; Reinhard et al. 2019). Consistent with this physiological profile, WF cells are required to detect prey within the visual field of the mouse (Hoy, Bishop, and Niell 2019).

NF cells, in contrast, extend a narrow column of dendrites vertically through sSC and have a correspondingly small spatial receptive field (~100 deg²) and preference for small stimuli (Gale and Murphy 2014; Hoy, Bishop, and Niell 2019). NF cells respond robustly to faster speeds than WF cells and often exhibit DS tuning that is likely inherited from putative inputs from ooDSGCs that complement other contrast-encoding inputs (Hoy, Bishop, and Niell 2019; Reinhard et al. 2019; Gale and Murphy 2014). Unlike WF cells, NF cells are not required for mice to
consistently detect insect prey. However, silencing NF cells impedes the ability of a mouse to 1) maintain its prey within its binocular visual field during pursuit and 2) reliably intercept or capture prey (Hoy, Bishop, and Niell 2019).

The visual features that NF cells encode are consistent with insect prey (i.e., a small object that can move relatively quickly) (Hoy, Bishop, and Niell 2019). How the optimal response properties of NF cells (i.e., small, fast, DS) contribute to this role in predation has not been tested. However, given the projections of NF cells to deep layers of SC and to the parabigeminal nucleus (Reinhard et al. 2019; Gale and Murphy 2014), it is likely that the NF cells are involved in generating the motor commands necessary to orient the mouse toward a salient feature of visual space (i.e., prey). Chapters 2 and 3 of this dissertation explore the contributions of DS and contrast-encoding inputs to sSC – including to NF cells – and how they can help inform predatory behaviors.

1.5 Scope of the dissertation

Functional and anatomical characterization of cell types in the retina have revealed the participation of stereotyped cell types in distinct circuits that encode aspects of the visual scene. Despite a basic understanding of the connectivity of retinal circuits, in many cases, the functional significance of specific retinal circuits or components thereof remains unclear. This dissertation evaluates the contributions of retinocollicular pathways encoding either 1) motion direction (Chapter 2) or 2) the binocular visual field (Chapter 3) to predation.

1.6 References


Chapter 2: Direction Selectivity and Predation in Mice

2.1 Introduction

Despite their position in the food chain and their relatively lateral-facing eyes characteristic of prey animals, mice – including the canonical C57BL/J6 lab strain – can engage in predatory behaviors (Hoy et al. 2016). To do so, mice rely on vision to detect, pursue, and capture insects, requiring the convergence of retinal information from both eyes in retinorecipient brain areas (i.e., binocular vision; (Johnson et al. 2021)). Retinal ganglion cells (RGCs) project to more than 50 brain areas (Martersteck et al. 2017). Among these projections, superior colliculus (SC) has previously been implicated in predation (Shang et al. 2019; Zhao et al. 2019; Hoy, Bishop, and Niell 2019).

The optogenetic activation of SC neurons that project to the subthalamic nucleus (STN) is sufficient to trigger predation, reducing the latency of prey approaches and attacks (Shang et al. 2019). These SC neurons and their postsynaptic partners in the zona incerta region of STN convert visual and tactile sensory information into a behavioral output (Shang et al. 2019; Zhao et al. 2019). Another study that investigated the contributions of genetically defined subtypes of neurons in SC to predation revealed that specialized cell types in the superficial layer of SC (sSC) perform different functions in hunting, ranging from detection to pursuit and capture (Hoy, Bishop, and Niell 2019). Notably, when the population of narrow-field (NF) cells in sSC was
pharmacogenetically silenced, mice exhibited deficits in orienting toward, pursuing, and catching prey (Hoy, Bishop, and Niell 2019).

NF cells encode multiple stimulus features, including stimulus size, speed, and direction of motion, independent of contrast (Hoy, Bishop, and Niell 2019). How these stimulus features are used to guide predation remains unknown. Mice are most likely to approach objects that occupy <10° of visual space and move at <50°/s (Procacci et al. 2020). These parameters describe the size and speed of prey (i.e., crickets, (Johnson et al. 2021)) and elicit robust responses in NF cells in sSC (Hoy, Bishop, and Niell 2019). Mice often fail to approach or freeze in response to prey-like stimuli that are >10° of visual space or that move faster than 50°/s (Procacci et al. 2020), suggesting that size and speed might determine the extent to which prey is appetitive to a mouse, rather than the ability of the mouse to accurately pursue it. As mice prefer moving over stationary prey (Shang et al. 2019), how prey moves (i.e., motion direction) might be a key determinant of the accuracy and precision with which mice respond. This has not been tested.

Motion direction is computed in the retina and encoded in the spike trains of direction-selective ganglion cells (DSGCs) that innervate sSC, the dorsolateral geniculate nucleus shell (dLGN; (Huberman et al. 2009; Kay et al. 2011; Rivlin-Etzion et al. 2011)) and nuclei of the accessory optic system (AOS; (Oyster et al. 1980; Simpson 1984; Dhande et al. 2013)). Direction-selective inputs to the AOS, mediated exclusively by ON-responsive DSGCs (oDSGCs), are known to drive gaze-stabilizing eye movements (i.e., optokinetic reflex) in response to global motion (Yoshida et al. 2001; Yonehara et al. 2016), whereas neurons in layers 2/3 of primary visual cortex (V1) can compute direction of motion de novo, independent of upstream inputs from ON/OFF-responsive DSGCs (ooDSGCs) to dLGN. The direction-selective responses of neurons
in sSC, such as NF cells, are dependent on innervation from oODSGCs (Shi et al. 2017), yet the functional significance of this input remains unknown.

Recently, a tri-synaptic circuit encompassing oODSGCs, sSC neurons, and parabigeminal neurons was described (Reinhard et al. 2019). Among the sources of SC input to parabigeminal neurons are NF cells (Gale and Murphy 2014), which are direction-selective and therefore likely receive input from oODSGCs. Of note, the deep layers of SC and collicular outputs to the parabigeminal nucleus have been implicated in generating orienting head movements toward salient visual stimuli and saccade-like eye movements in response to moving stimuli, respectively (Ma et al. 2013; Sahibzada, Dean, and Redgrave 1986). These pieces of evidence suggest a potential role of direction-selective signaling in the NF cells of sSC, mediated by oODSGC retinal input, in the generation of orienting head and eye movements toward behaviorally relevant stimuli, such as prey.

Here, we test for the role of direction selectivity in predation by manipulating the brain and retinal circuits that encode this feature in mice and assessing their ability to approach, pursue, and capture moving prey efficiently.

2.2 Method

Animals

The procedures in this study were approved by the Animal Studies Committee of Washington University School of Medicine (Protocol #20200055) and performed in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.
ChAT-Cre mice (Rossi et al. 2011) were used to target genetic manipulations to the starburst amacrine cells (SACs) in the retina. For SAC ablation experiments, ChAT-Cre mice were crossed to a strain in which the diphtheria toxin receptor is expressed in a Cre-dependent manner (DTR mice; (Buch et al. 2005)). For a subset of targeted patch-clamp experiments, ChAT-DTR mice were crossed to DRD4-EGFP mice, in which ooDSGCs preferring posterior motion direction express GFP (Huberman et al. 2009). GRP-Cre mice were used to manipulate NF cells in sSC (Gerfen, Paletzki, and Heintz 2013). Wildtype (C57BL/6J), ChAT-Cre, or GRP-Cre mice were used for control experiments in which the AOS or V1 were manipulated.

Experiments were performed using adult mice (postnatal day 45 [P45] and older) of both sexes. Mice were housed on a 12 h light/12 h dark cycle. Non-food restricted mice were provided with *ad libitum* access to food and water. Food pellets were removed 16-20 h prior to testing predation.

**Surgical procedures**

For all surgical procedures, mice were anesthetized by intraperitoneal (i.p.) injection of ketamine/xylazine cocktail (0.1 mg/10 g). Meloxicam (4 mg/kg) was administered post-operatively by subcutaneous injection at 0, 24, and 48 hours. The scalp was sanitized with betadine solution and 70% ethanol before incisions were made to expose the cranial bone for craniotomy or head-plate attachment.

For behavioral experiments, viruses encoding inhibitory DREADDs, caspase3, or fluorophores were delivered by stereotactic injection to sSC, nuclei of the AOS and V1, as described, below. For Rabies-mediated tracing experiments, a series of 2 virus injections was performed to introduce both helper proteins and the Rabies virus, as described, below. A nanoinjector
(Nanoject III, Drummond Scientific) fitted with a pulled glass pipette was used to deliver ~100-200 nL of virus to stereotactic coordinates determined relative to Bregma. Surgical sites healed and staples were removed 7-10 days after injections.

**Head-fixation for behavioral experiments**

Prior to optokinetic reflex experiments, mice were outfitted with custom titanium bars (eMachineShop.com) to permit head-restraint during visual stimulation. The periosteum was removed from the exposed skull with a scalpel and application of 3% H$_2$O$_2$ solution. After washing the skull with 1X PBS, the skull was thoroughly dried and abraded with a scalpel to improve dental cement adhesion. Titanium bars were then fixed to the mouse skull using tissue adhesive (Vetbond, 3M) and dental acrylic (C&B Metabond, Parkell). After head-plate insertion, mice recovered for at least 48 hours before beginning behavioral experiments.

**Virus delivery for behavioral experiments**

Virus injections were performed 4 weeks prior to behavioral testing (i.e., at least 3 weeks prior to the onset of training). The expression of inhibitory DREADDs (iDREADDs) was targeted genetically and/or anatomically to neurons in sSC, AOS, or V1 to determine the relative contributions of direction-selective (DS) visual pathways in the brain to predatory and other visual behaviors. To control for any off-target, acute effects of iDREADD-based manipulations, genetically defined cells in sSC were ablated by inducing caspase3-mediated apoptosis (Yang et al. 2013). To control for any off-target effects of CNO application, the expression of a fluorescent tag (i.e., GCaMP) was targeted to sSC.
Viruses encoding Cre-dependent iDREADDs, a fluorophore, or the caspase3 protein were injected bilaterally to the sSC of GRP-Cre mice, via 2 craniotomies over either hemisphere (4 injection sites, each hemisphere). iDREADDs viruses (AAV8-hSyn-DIO-HA-hM4D(Gi)-IRES-mCitrine or AAV PHP.eB-hSyn-DIO-hM4D(Gi)-mCherry) were generous gifts from Bryan Roth (Addgene viral preps #50455 and #44362-PHPeB, respectively)(Krashes et al. 2011). The GCaMP6s virus (AAV9-Syn-FLEX-GCaMP6s) was a gift from Douglas Kim and the GENIE Project (Addgene viral prep#100843-AAV9) (Chen et al. 2013). The caspase3 virus (AAV5-EF1a-taCasp3-TEVp) was a gift from Nirao Shah and Jim Wells (Addgene viral prep #45580-AAV5) (Yang et al. 2013).

To target AOS, viruses to constitutively express iDREADDs were injected bilaterally to the medial terminal nucleus (MTN), the nucleus of the optic tract (NOT), and the dorsal terminal nucleus (DTN) of wildtype, ChAT-Cre, or GRP-Cre mice. Similarly, viruses to constitutively express iDREADDs were delivered bilaterally to V1 via 2 craniotomies over either hemisphere (5 injection sites, each hemisphere). The constitutively active iDREADDs virus (AAV5-Syn-hM4D(Gi)-mCherry) was a gift from Bryan Roth (Addgene viral prep #50475).

**Rabies-mediated retrograde tracing**

The sSC of GRP-Cre mice was injected unilaterally via a large craniotomy over sSC (4 injection sites) with a Cre-dependent helper AAV (AAV8-hSyn-FLEX-TVA-P2A-GFP-2A-oG). The helper AAV encodes an envelope protein (avian tumor virus receptor A, TVA) flagged with GFP, required for the Rabies virus to enter cells, and the glycoprotein (G) required for Rabies to retrogradely transmit to cells immediately presynaptic to TVA receptor- and G protein-expressing GRP-Cre+ neurons (Wickersham et al. 2007). Following the injection of the helper
virus, mice recovered for at least 4 weeks to allow robust virus expression. After this point, the incision was re-opened, and the G-deleted Rabies virus flagged with mCherry (EnVA G-deleted Rabies-mCherry) was injected to the same stereotactic coordinates that received the helper virus injections.

With this injection scheme, the mCherry-tagged G-deleted Rabies virus entered only GRP-Cre+ cells that virally expressed the GFP-tagged TVA receptor and G protein. Thus, double-positive GFP/mCherry+ neurons could be identified as starter cells, from which the mCherry-tagged Rabies virus could transmit to the cells immediately pre-synaptic (i.e., mCherry+ cells). Rabies has been shown to be neurolytic, therefore, its expression was limited to 4-7 days post-injection. During this window, 2-photon guided patch clamp experiments were performed, as described below, to characterize the RGC types that directly innervate GRP-Cre+ NF cells in sSC.

_SAC ablation_

Mice were anesthetized by i.p. injection of ketamine/xylazine cocktail (0.1 mg/10 g). Diphtheria toxin (DT; 1.6 ng in 2 µL PBS) was then delivered bilaterally to ChAT-DTR mice by intraocular injection (Nanoject II, Drummond Scientific) to selectively ablate SACs using a protocol modified from Hillier et al. (2017). To determine the time course of the loss of SAC function, the optokinetic reflex was measured on each of the 9 days following intraocular injection. To determine the time course of cell death, ChAT-DTR mice were sacrificed daily, beginning 3 days after intraocular injection, and retinas were prepared for immunohistochemistry. To control for genotype and/or DT injection, these experiments were performed in ChAT-DTR mice that received a 2 µL PBS injection to both eyes and/or ChAT-DTR littermates that did not express Cre and received a 2 µL DT injection to both eyes.
Predation was assayed using a protocol similar to those described by Hoy et al. (2016) and Johnson et al. (2021). Forty-eight hours before training began, mice were singly housed, acclimated to handling, and 2-3 crickets were introduced to each cage in addition to food pellets. Mice were placed on a food-restricted diet 16-20 hours before training, food pellets were removed from the cages, and each mouse was given 2-3 crickets. On the first day of training, mice were introduced to the behavioral arena (width: 45 cm, length: 38 cm, height: 30 cm, with padded flooring) and given 3 minutes to acclimate. A cricket was then placed in the arena, and mice were given up to 5 minutes to capture the prey. After 5 minutes or after the successful capture of the cricket, the arena was cleaned, and a new cricket was introduced until each mouse had the opportunity to capture 3 crickets. The interactions between each mouse and cricket were recorded with an overhead camera (C310, Logitech). Following 3 trials, each mouse was returned to its home cage and given access to food pellets for 4-6 hours. Food pellets were removed from each cage 16-20 hours prior to the subsequent training day. Mice were trained for 4-5 days until the behavior plateaued, prior to entering the testing phase of the behavior.

Mice expressing iDREADDs or GCaMP from viral injections received PBS i.p. injections on days 4, 5, and 6. These mice received CNO i.p. injections (1 mg/kg) on day 6. All behavioral results are reported from CNO- and PBS-injected trials spaced at least 4 hours apart on day 6 to minimize day-to-day variability within mice.

ChAT-DTR mice received 4 days of training, and behavioral results are reported from the test on day 5. SAC-ablation experiments revealed a stereotyped time course for the loss of function and cell death by 10 days after intraocular DT injections. Therefore, predation experiments with
ChAT-DTR mice were performed with cell death completed prior to behavioral training (training phase manipulation) or coinciding with the test (testing phase manipulation). For the training phase manipulation, ChAT-DTR mice began training more than 10 days after intraocular DT injection. For the testing phase manipulation, ChAT-DTR mice began training 5 days after intraocular DT injection, such that testing occurred 11 days after DT injection. ChAT-DTR mice that received a testing phase manipulation were sacrificed immediately after behavioral testing was complete and retinal tissue was prepared for immunohistochemistry.

**Video analysis of predation data**

Overhead recordings of mouse-cricket interactions were analyzed, as previously described by Johnson et al. (2021). The cricket and mouse’s ears, nose, and tail base were tracked across all video frames using DeepLabCut (Mathis et al. 2018; Nath et al. 2019) and manually edited using custom software (OpenCV, Python). The head position of the mouse was extrapolated from the midpoint of its ears, and this point was used to calculate the distance to the cricket and the speed of the mouse. The heading of the mouse was defined by the vector from the head position to the nose. The angle between the heading of the mouse and the vector between the mouse’s head and cricket defined the azimuth during the approach and contact phases.

Based on our previous characterization that mice perform a stereotyped bite-and grab sequence when close enough to prey (Johnson et al. 2021), we defined behavioral epochs in which the mouse was within 4 cm of prey as a contact. We defined periods in which the mouse ran toward prey at speeds >10 cm/s to reduce its distance from the cricket by >7 cm/s as approaches. Approaches ended when these criteria were no longer satisfied or when the mouse contacted the cricket. We defined the approach interval as the amount of time between the end of an approach
or contact and initiation of a subsequent approach to estimate detection latency. Approaches frequently preceded contacts and contacts preceded capture. We determined the probability of converting approaches to contacts, as follows:

\[
p(\text{contact} | \text{approach}) = \frac{\text{# contacts within } 250 \text{ ms of an approach ending}}{\text{total # of approaches}}
\]

We determined the probability of converting contacts to capture, as follows:

\[
p(\text{capture} | \text{contact}) = \frac{1}{\text{# contacts}}
\]

Capture times, approach intervals, probabilities of converting approaches to contacts or contacts to capture, and azimuth are reported for all conditions. Cumulative density plots show these data for all trials. Bar plots show these data averaged across 3-4 trials per mouse.

**Optokinetic reflex recording and analysis**

Mice had head-plates implanted more than 48 hours prior to and were dark-adapted for at least an hour before behavioral testing. Mice expressing iDREADDs or GCaMP from viral injections received an i.p. injection of CNO (1 mg/kg) or PBS 5 minutes before testing and then were restrained in a custom head-fixed holder before exposure to visual stimuli. ChAT-DTR mice were head-fixed directly. Pupil size and eye movements were tracked and recorded under infrared illumination of the left eye using the ETL-200 eye-tracking system (ISCAN). To assess the optokinetic reflex, square-wave gratings (0.05 cycles/°) at varying Michelson contrasts (10%, 20%, 50%, 100%) moving at 10°/s in the temporal-nasal and/or upward direction were presented on a monitor placed 11 cm from the mouse’s left eye at a 45° angle. Each stimulus presentation consisted of 10 s of a uniform grey screen, 60 s of drifting gratings, followed by 10 s of grey
screen. Each stimulus presentation was repeated twice for each mouse. Eye-tracking movements (ETMs) were quantified as the number of saccade-like eye movements preceded by a slow tracking motion (Cahill and Nathans 2008).

**Visual cliff**

Visual cliff tests were performed, as previously described by T. Kim et al. (2020), to assess depth perception using a 56-cm by 41-cm platform (width x depth) with a 3.8-cm by 3.4-cm ridge (width x height) across its center. On one side of the ridge, a checkered pattern was immediately below the platform (i.e., the shallow side), and on the other side, an identical checkered pattern was 61 cm below the platform (i.e., the cliff side). On subsequent days, mice expressing iDREADDs or GCaMP from viral injections received i.p. injections of CNO (1 mg/kg) and PBS 5 minutes before behavioral testing. ChAT-DTR mice required only 1 day of behavioral testing and these mice did not receive i.p. injections. Mice were then placed on the ridge and filmed via a USB camera (C310, Logitech). For each mouse, under each condition, we measured the percentage of shallow-side choices in 10 trials of the behavior.

**Tissue preparation**

Mice were deeply anesthetized with CO₂ and killed by cervical dislocation. Brains of mice that received stereotactic virus injections were harvested and fixed in 4% paraformaldehyde (PFA) overnight. For immunohistochemistry experiments, the eyes of ChAT-DTR mice were removed and transferred to oxygenated mACSF_{HEPES} containing (in mM): 119 NaCl, 2.5 KCl, 1 NaH₂PO₄, 2.5 CaCl₂, 1.3 MgCl₂, 20 HEPES and 11 glucose (pH adjusted to 7.37 using NaOH). Retinas were either isolated and flat-mounted on filter paper (Millipore) or left in the eyecup for fixation with 4% PFA for 20 minutes. For electrophysiology experiments, mice were dark-adapted for
more than 1.5 hours and enucleated under dim-red light. Retinas were isolated under infrared illumination (>900 nm) in oxygenated mACSF NaHCO₃ containing (in mM): 125 NaCl, 2.5 KCl, 1 MgCl₂, 1.25 NaH₂PO₄, 2 CaCl₂, 20 glucose, 26 NaHCO₃ and 0.5 L-glutamine equilibrated with 95% O₂/5% CO₂. Retinas were flat-mounted ganglion cell side-up on transparent membrane discs (13 mm, Whatman Anodisc).

**Immunohistochemistry**

Vibratome slices of brains (thickness 100 µm) were prepared covering regions of interest (sSC, AOS nuclei, V1). Retina cups were embedded in 4% low-melt agarose and vibratome slices (thickness 60 µm) were prepared. Retina or brain slices were blocked in 10% normal donkey serum (NDS) in PBS for 2 hours. Slices were then incubated in for 2 days in primary antibody at 4°C. Slices were washed 3 times in PBS for 30 minutes and incubated in secondary antibodies for 2 hours.

Flat-mounted retinas were cryoprotected (1 hour 10% sucrose in PBS, 1 hour 20% sucrose in PBS, overnight 30% sucrose in PBS at 4°C) and then frozen and thawed 3 times. Retinas were then blocked with 10% NDS in PBS for 2 hours, incubated in primary antibodies for 5 days at 4°C and washed 3 times in PBS for 1 hours. Retinas were subsequently incubated in secondary antibodies for 2 days.

A goat anti-ChAT antibody was used to label SACs in the retina and a rabbit anti-Ds-Red antibody was used to amplify retrogradely labeled NF-projecting RGCs. Primary antibodies were conjugated to Alexa 488, 568 or 633 for detection. Nuclei were labeled with DAPI to determine retina or brain anatomy.
Electrophysiology

Whole-cell patch-clamp recordings were obtained from the dorsal halves of dark-adapted, flat-mounted retinas. Retinas were perfused (~3 mL/min) with warm (~32°C) mACSF\textsubscript{NaHCO\textsubscript{3}}. Fluorescently labeled DRD4-EGFP\textsuperscript{+} ooDSGCs or mCherry-labeled RGCs from retrograde tracing experiments were targeted for recording under 2-photon guidance (Mai Tai DeepSee, Spectra-Physics). Patch pipettes were pulled from borosilicate glass using a P97 puller (Sutter Instruments) and had resistances of 4-6 M\textOmega. The intracellular solution for current-clamp recordings contained (in mM): 125 K-glucuronate, 10 NaCl, 1 MgCl\textsubscript{2}, 10 EGTA, 5 HEPES, 5 ATP-Na\textsubscript{2} and 0.1 GTP-Na (pH adjusted to 7.2 with KOH).

Quantification and statistical analysis

No statistical methods were used to predetermine sample sizes. P values were calculated using Mann-Whitney U tests, Kruskal-Wallis tests or bootstrapping and were used to assess the statistical significance of observed differences. Population data are reported as mean ± SEM, and N represents the numbers of animals or cells analyzed.

2.3 Results

Direction-selective cells in sSC are necessary for efficient predation

Previous reports have implicated the sSC in predatory mouse behaviors (Shang et al. 2019; Hoy, Bishop, and Niell 2019). Here, we tested the contributions of a subpopulation of sSC cells that encode motion direction, small stimulus size and rapid movement, independent of contrast to hunting. To accomplish this, we expressed inhibitory DREADDs (iDREADDs) specifically in NF cells by performing stereotactic injections of AAVs encoding Cre-dependent iDREADDs
into the sSC of GRP-Cre mice. At least 3 weeks after receiving brain injections, the mice were food-restricted and trained to hunt live crickets.

For predation experiments, mice were food-restricted 16-18 hours before the onset of behavioral testing to increase their motivation to interact with live prey. The mice underwent a 4–5-day training period in which they habituated to hunting crickets in a brightly lit arena. The performance of the mice steadily improved for 3-4 days before plateauing, at which point their performance was tested across 4 test trials. All test trials were analyzed using sophisticated behavioral tracking algorithms to extract information about the relative interactions between the mouse and cricket (see Method for details).

Once hunting performance plateaued, mice received i.p. injections of PBS or clozapine-N-oxide (CNO) to activate the iDREADDs and inhibit the population of NF cells expressing the iDREADDs. All mice received both PBS and CNO i.p. injections on the same test day, such that the reversible effect of activating the iDREADDs in a single mouse could be tested. Silencing NF cells resulted in a robust deficit in hunting, represented by increased total capture time, delays in initiating new approaches, deficits in converting approaches to contacts and converting contacts to captures, and impaired bearing toward the cricket during approaches (Figure 2.1). Notably, these observed deficits were all reversible between the application of either CNO or PBS by i.p. injection to the same mouse on the same day, therefore implicating NF cells in predation, as was previously reported.
Figure 2.1. Chemogenetic silencing of NF cells in sSC impairs predation. A) Experimental schedule. Bilateral stereotactic injections of an adeno-associated virus (AAV) encoding Cre-dependent hM4D(i) (iDREADDs) were performed to sSC of GRP-Cre mice. After at least 3 weeks of virus expression, mice were food-restricted and trained to hunt crickets in an arena. Behavioral performance typically plateaued after 4-5 days of training. At test, mice received an i.p. injection of PBS or CNO to inhibit neurons expressing virally delivered iDREADDs. All mice received both PBS and CNO i.p. injections. B) Representative hunting overviews of a mouse injected with PBS (grey) or CNO (magenta) pursuing a cricket (black). C1-F1) Cumulative density plots generated for all trials performed, pooled across all mice tested. N = 85 PBS and N = 85 CNO trials. C1) Time from introduction of a cricket to its capture (PBS = 14.92±1.69, CNO = 44.22±5.62, p<0.00001). D1) Latency to detect prey and initiate new approaches (PBS = 1.10±0.14, CNO = 2.62±0.40, p<0.0001). E1) Probability that mice successfully convert approaches into contacts (PBS = 0.76±0.02, CNO = 0.67±0.02, p<0.05). F1) Probability that mice successfully convert contacts into captures (PBS = 0.36±0.04, CNO = 0.19±0.02, p<0.001). G1) Probability distribution of cricket azimuth during approaches or contacts across all trials (p>0.05). C2-G2) Summary plots for all mice tested. Each data point represents an average of 3-4 trials per condition for each mouse. N = 21 mice. C2) Time from introduction of a cricket to its capture (PBS = 14.69±2.52, CNO = 45.62±9.69, p<0.01). D2) Latency to detect prey and initiate new approaches (PBS = 1.10±0.18, CNO = 2.66±0.55, p<0.05). E2) Probability that mice successfully convert approaches into contacts (PBS = 0.77±0.04, CNO = 0.67±0.04, p=0.07). F2) Probability that mice successfully convert contacts into captures (PBS = 0.36±0.05, CNO = 0.19±0.03, p<0.05). G2) Mean cricket azimuth during approaches or contacts (PBS = 24.76±3.79, CNO = 34.52±3.08, p<0.05).

We confirmed this result by testing whether the observed deficit could be attributed off-target effects of CNO administration. We performed bilateral sSC injections of a Cre-dependent genetically encoded calcium indicator (GCaMP) in littermate GRP-Cre mice. At least 4 weeks after virus injections, we food-restricted and trained the mice to hunt, as described. On test day, we administered both CNO and PBS i.p. injections to these mice prior to test trials. We observed
no difference between CNO and PBS conditions in the capture time, probabilities of converting approaches to contacts or contacts to captures, or in the bearing toward the cricket during approach (Figure 2.2). Thus, neither the perturbation of sSC neurons by the introduction of a virus nor the administration of CNO are sufficient to induce the predation deficiencies observed following the pharmacogenetic silencing of NF cells in sSC.

Figure 2.2. CNO administration does not induce predatory deficits in the absence of iDREADDs expression. A) Experimental schedule. Bilateral stereotactic injections of an AAV encoding Cre-dependent GCaMP were performed to sSC of GRP-Cre mice. Behavioral training began at least 3 weeks after virus injections. At test, mice received an i.p. injection of PBS or CNO. All mice received both PBS and CNO i.p. injections. B) Representative hunting overview of a mouse injected with PBS (grey) or CNO (teal) pursuing a cricket (black). C1-F1) Cumulative density plots generated for all trials performed, pooled across all mice tested. N = 45 PBS and N = 48 CNO trials. C1) Time from introduction of a cricket to its capture (PBS = 13.35±1.50, CNO = 14.92±1.67, p>0.05). D1) Latency to detect prey and initiate new approaches (PBS = 0.91±0.12, CNO = 0.82±0.08, p>0.05). E1) Probability that mice successfully convert approaches into contacts (PBS = 0.77±0.03, CNO = 0.79±0.03, p>0.05). F1) Probability that mice successfully convert contacts into captures (PBS = 0.36±0.06, CNO = 0.30±0.05, p>0.05). G1) Probability distribution of cricket azimuth during approaches or contacts across all trials (p>0.05). C2-G2) Summary plots for all mice tested. Each data point represents an average of 3-4 trials per condition for each mouse. N = 12 mice. C2) Time from introduction of a cricket to its capture (PBS = 12.89±2.19, CNO = 14.92±2.56, p>0.05). D2) Latency to detect prey and initiate new approaches (PBS = 0.88±0.18, CNO = 0.82±0.10, p>0.05). E2) Probability that mice successfully convert approaches into contacts (PBS = 0.78±0.04, CNO = 0.79±0.15, p>0.05). F2) Probability that mice successfully convert contacts into captures (PBS = 0.37±0.07, CNO = 0.30±0.05, p>0.05). G2) Mean cricket azimuth during approaches or contacts (PBS = 30.00±5.26, CNO = 27.50±0.75, p>0.05).
Previous studies have suggested that transient circuit manipulations, for example, by optogenetic stimulation or pharmacogenetic silencing, can induce large, non-physiological circuit and behavioral aberrations that can be mistakenly attributed to a particular computation in a pathway (Otchy et al. 2015). To discount this possibility, we ablated NF cells in a separate cohort of mice. We injected the sSC of a separate cohort of GRP-Cre mice bilaterally with a Cre-dependent AAV that encoded caspase3, which induces apoptosis upon expression. More than 3 weeks after virus injections, we food-restricted and trained these mice to hunt crickets. Relative to GRP-Cre mice injected bilaterally with GCaMP, the Caspase3-injected mice failed to initiate new approaches and accurately pursue crickets (Figure 2.3). The effect of ablating NF cells in sSC approximately phenocopied the deficits induced by pharmacogenetic silencing, further suggesting that the CNO-induced deficits observed stem from a key computation performed by NF cells.
Figure 2.3. NF cell ablation phenocopies pharmacogenetic silencing. A) Experimental schedule. Bilateral stereotactic injections of an AAV encoding Cre-dependent Caspase3 were performed to sSC of GRP-Cre mice. Behavioral training began at least 3 weeks after virus injections. Mice were tested directly once performance plateaued. Control data reported here are from GRP-Cre mice with GCaMP injections to sSC and PBS i.p. injections at test. Control data are reproduced from Figure 2.2. B) Representative hunting overviews of mice that received either GCaMP (grey) or Caspase3 (magenta) stereotactic injections pursuing a cricket (black). C1-F1) Cumulative density plots generated for all trials performed, pooled across all mice tested. N = 45 control and N = 25 caspase3 trials. C1) Time from introduction of a cricket to its capture (Control = 13.35±1.50, Caspase3 = 65.61±7.04, p<0.00001). D1) Latency to detect prey and initiate new approaches (Control = 0.91±0.12, Caspase3 = 4.52±0.49, p<0.00001). E1) Probability that mice successfully convert approaches into contacts (Control = 0.77±0.03, Caspase3 = 0.54±0.03, p<0.001). F1) Probability that mice successfully convert contacts into captures (Control = 0.36±0.06, Caspase3 = 0.13±0.01, p<0.01). G1) Probability distribution of cricket azimuth during approaches or contacts across all trials (p>0.05). C2-G2) Summary plots for all mice tested. Each data point represents an average of 3-4 trials per condition for each mouse. N = 7 caspase3 and N = 12 control mice. C2) Time from introduction of a cricket to its capture (Control = 12.89±2.19, Caspase3 = 67.22±9.10, p<0.0001). D2) Latency to detect prey and initiate new approaches (Control = 0.88±0.18, Caspase3 = 4.55±0.50, p<0.0001). E2) Probability that mice successfully convert approaches into contacts (Control = 0.78±0.04, Caspase3 = 0.54±0.04, p<0.01). F2) Probability that mice successfully convert contacts into captures (Control = 0.37±0.07, Caspase3 = 0.12±0.01, p<0.01). G2) Mean cricket azimuth during approaches or contacts (Control = 30.00±5.26, Caspase3 = 27.14±3.06, p>0.05).

Direction-selective V1 and AOS pathways make negligible contributions to predation

NF cells integrate visual information from different RGC types with different tuning characteristics and provide input to a behavioral pathway that relies on attentional cues and generates complex eye, head, and body movements in response to a stimulus of interest (Gale
and Murphy 2014). Our NF cell silencing and ablation data show a clear role of NF cells in initiating predatory behaviors. However, whether NF cells rely on key pieces of visual information (i.e., the presence of a visual feature) or only need sufficient excitatory drive (i.e., from any source) to begin initiating motor commands, for example, remains unknown.

When considered as a visual stimulus, crickets are small (~6°) dark objects that move quickly (<50°/s) in a particular direction (Johnson et al. 2021). Each of these features is represented by the optimal response properties that have been reported for NF cells (Hoy, Bishop, and Niell 2019). Given that mice must respond reliably to the movement trajectories of crickets in real time, we reasoned that early sensory processing of stimulus direction of motion (i.e., direction selectivity) might be important for generating the eye, head and body movements necessary to pursue, approach, contact and capture live prey.

We first tested the necessity of direction selective (DS) signaling in visual pathways outside of sSC to determine whether this might be a generalizable principle for how this type of information is utilized in the brain. To accomplish this, we targeted 2 pathways that receive DS information directly from the retina and/or that compute direction selectivity de novo.

Like sSC, dLGN receives directional information from ooDSGCs in its shell region (Huberman et al. 2009; Kay et al. 2011; Rivlin-Etzion et al. 2011), where select thalamocortical cells encode motion direction and project to layer 4 of the primary visual cortex (V1) to inform visual processing (Douglas and Martin 2004). Previous studies have shown that non-DS inputs to the dLGN-V1 pathway are sufficient to compute DS de novo in layers 2/3 of V1 (Hillier et al. 2017). Therefore, to eliminate DS signaling in this pathway, we targeted V1 for iDREADD-based silencing experiments.
We injected AAVs encoding constitutively active iDREADDs bilaterally to the V1 of wildtype, ChAT-Cre or GRP-Cre mice to silence all neurons within the anatomical bounds of our injections, regardless of their cell type identity. At least 3 weeks after virus injections, we tested the performance of these mice on the hunting assay, testing each mouse with both PBS and CNO i.p. injections on the same day, after performance had plateaued. Global silencing of V1 had no effect on predation, as all mice efficiently detected, approached, captured, and consumed their crickets under both PBS and CNO conditions (Figure 2.4).

Figure 2.4. Predation persists following chemogenetic silencing of V1. A) Experimental schedule. Bilateral stereotactic injections of an AAV encoding constitutively active iDREADDs were performed to the V1 of wildtype, ChAT-Cre or GRP-Cre mice. Behavioral training began more than 3 weeks after injection. At test, mice received an i.p. injection of PBS or CNO. All mice received both PBS and CNO i.p. injections. B) Representative hunting overviews of a mouse injected with PBS (grey) or CNO (peach) pursuing a cricket (black). C1-F1) Cumulative density plots generated for all trials performed, pooled across all mice tested. N = 20 PBS and N = 22 CNO trials. C1) Time from introduction of a cricket to its capture (PBS = 18.55±3.92, CNO = 17.30±2.62, p>0.05). D1) Latency to detect prey and initiate new approaches (PBS = 1.23±0.21, CNO = 1.18±0.17, p>0.05). E1) Probability that mice successfully convert approaches into contacts (PBS = 0.64±0.05, CNO = 0.62±0.04, p>0.05). F1) Probability that mice successfully convert contacts into captures (PBS = 0.20±0.03, CNO = 0.22±0.04, p>0.05). G1) Probability distribution of cricket azimuth during approaches or contacts across all trials (p>0.05). C2-G2) Summary plots for all mice tested. Each data point represents an average of 3-4 trials per condition for each mouse. N = 6.
mice. C2) Time from introduction of a cricket to its capture (PBS = 19.56±3.50, CNO = 18.07±3.15, p>0.05). D2) Latency to detect prey and initiate new approaches (PBS = 1.30±0.25, CNO = 1.22±0.22, p>0.05). E2) Probability that mice successfully convert approaches into contacts (PBS = 0.63±0.04, CNO = 0.62±0.03, p>0.05). F2) Probability that mice successfully convert contacts into captures (PBS = 0.20±0.03, CNO = 0.22±0.03, p>0.05). G2) Mean cricket azimuth during approaches or contacts (PBS = 20.83±3.00, CNO = 25.00±4.47, p>0.05).

We likewise tested the necessity of the visual pathway consisting of the nuclei of the AOS (i.e., MTN, NOT, and DTN), which receives input directly from oDSGCs to mediate gaze stabilization (Dhande et al. 2013; Oyster et al. 1980; Simpson 1984; Yonehara et al. 2016; Yoshida et al. 2001). We injected all AOS nuclei bilaterally in WT, ChAT-Cre or GRP-Cre mice with an AAV encoding constitutively active iDREADDs to silence all neurons within the anatomical bounds of our injections. At least 4 weeks after virus injections, we tested the hunting performance of these mice and found that signaling by AOS nuclei is not required for predation (Figure 2.5).
injection of PBS or CNO. All mice received both PBS and CNO i.p. injections. B) Representative hunting overviews of a mouse injected with PBS (grey) or CNO (purple) pursuing a cricket (black). C1-G1) Cumulative density plots generated for all trials performed, pooled across all mice tested. N = 16 PBS and N = 16 CNO trials. C1) Time from introduction of a cricket to its capture (PBS = 8.51±1.89, CNO = 16.49±2.49, p<0.01). D1) Latency to detect prey and initiate new approaches (PBS = 0.86±0.22, CNO = 1.40±0.19, p<0.05). E1) Probability that mice successfully convert approaches into contacts (PBS = 0.73±0.06, CNO = 0.64±0.06, p>0.05). F1) Probability that mice successfully convert contacts into captures (PBS = 0.40±0.08, CNO = 0.26±0.06, p>0.05). G1) Probability distribution of cricket azimuth during approaches or contacts across all trials (p>0.05). C2-G2) Summary plots for all mice tested. Each data point represents an average of 3-4 trials per condition for each mouse. N = 4 mice. C2) Time from introduction of a cricket to its capture (PBS = 8.51±2.65, CNO = 16.49±3.36, p>0.05). D2) Latency to detect prey and initiate new approaches (PBS = 0.86±0.27, CNO = 1.40±0.21, p>0.05). E2) Probability that mice successfully convert approaches into contacts (PBS = 0.73±0.03, CNO = 0.64±0.08, p>0.05). F2) Probability that mice successfully convert contacts into captures (PBS = 0.40±0.08, CNO = 0.26±0.07, p>0.05). G2) Mean cricket azimuth during approaches or contacts (PBS = 22.50±4.79, CNO = 40.00±8.90, p>0.05).

Contributions of direction-selective visual pathways are specific and non-overlapping

To confirm the effectiveness and specificity of these manipulations, we tested all mice on the optokinetic reflex (OKR) and visual cliff assays (Figure 2.6). OKR is mediated by oDSGC projections to AOS nuclei to control gaze stabilization (Yoshida et al. 2001). Conversely, the visual cliff assay tests depth perception, which is mediated by the dLGN-V1 visual pathway. Therefore, given successful silencing manipulations of these various visual pathways, we expected target-specific deficits to these behaviors.

In response to silencing NF cells in sSC, predation was impaired; however, both OKR and visual cliff performances were left intact following the manipulation (Figure 2.6, top row). In response to dLGN-V1 silencing, only the visual cliff performance was impaired, as mice retained both OKR and ability to hunt (Figure 2.6, middle row). Finally, AOS silencing resulted in the loss or attenuation of both horizontal and vertical OKR, indicating that nuclei encoding both horizontal (DTN and NOT) and vertical (MTN) directions of motion were effectively silenced (Dhande et al. 2013). In contrast, both predation and visual cliff were normal in these mice (Figure 2.6, bottom row). All deficits were induced by that activation of the iDREADDs by CNO i.p. injection and were reversible with a counterbalanced PBS i.p. injection.
Figure 2.6. Discrete visual pathways drive distinct visual behaviors. Mice expressing iDREADDs bilaterally in sSC, V1 or AOS were tested on their ability to hunt live prey (i.e., predation), perceive depth (i.e., visual cliff), and maintain gaze stability across varying contrast levels (i.e., OKR). 

Top row: Behavioral performance of mice following pharmacogenetic silencing of NF cells in sSC. Left) Time from introduction of a cricket to its capture (p<0.01; N = 21 mice). Middle) Summary data of performance on a visual cliff test. For each mouse, the percentage of shallow-side choices in 10 trials was measured (p>0.05; N = 10 mice). Right) Quantification of eye-tracking movements (ETMs) elicited on an optokinetic test (p>0.05; N = 9 mice). 

Middle row: Behavioral performance, as in A1-3, of mice following pharmacogenetic silencing of V1. Left) Time from introduction of a cricket to its capture (p>0.05; N = 6 mice). Middle) Summary data of performance on a visual cliff test (p<0.05; N = 5 mice). Right) Quantification of ETMs (p>0.05; N = 5 mice). 

Bottom row: Behavioral performance, as in A1-3, of mice following pharmacogenetic silencing of the AOS nuclei. Left) Time from introduction of a cricket to its capture (p>0.05; N = 4 mice). Middle) Summary data of performance on a visual cliff test (p>0.05; N = 3 mice). Right) Quantification of ETMs (p<0.05; N = 4 mice).

The observed pattern of results revealed 3 main findings: 1) all target-specific silencing experiments were effective, 2) visual behaviors are governed specifically by visual pathways, and observed deficits are not due to global visual defects, and 3) if direction selectivity is important for responding effectively to prey, then it is a feature used specifically by neurons in sSC, not carried throughout all DS pathways in the brain.

NF cells in sSC are innervated by ooDSGCs

Circuitry linking ooDSGC projections to parabigeminal-projecting neurons in sSC was recently described (Reinhard et al. 2019). NF cells are known to be DS and to project to the parabigeminal nucleus (Gale and Murphy 2014). Given the overlap between these populations of
neurons in sSC, it is likely that NF cells receive input from ooDSGCs, yet the direct innervation of NF cells that are genetically defined in the GRP-Cre mice by ooDSGCs has yet to be described. We, therefore, sought to confirm an anatomical link between NF cells and ooDSGCs by performing Rabies-mediated retrograde tracing and 2-photon guided patch-clamp characterization of the RGC inputs to these cells.

We used a helper virus to express the GFP-tagged envelope protein, required for Rabies to inject cells, and G protein, required for Rabies to transmit presynaptically, in NF cells in sSC. At least 4 weeks later, we stereotactically injected the mCherry-tagged Rabies virus to the same coordinates to which we delivered the helper virus. NF cells expressing the helper virus that were injected by the Rabies virus were identified by dual expression of GFP and mCherry. Rabies transmitted one synapse presynaptic from these starter cells to express mCherry, allowing us to identify cells that directly innervated NF cells.

Our lab recently characterized the RGC types that constitute binocular vision by sending ipsilateral projections to subcortical targets such as sSC and dLGN. ooDSGCs are conspicuously absent from the 9 types that make up this population (Johnson et al. 2021). We therefore recorded RGCs in the retina contralateral to sSC Rabies injections. Among the many RGC types that synapse onto NF cells in sSC that have yet to be characterized, preliminary patch clamp experiments in these retinas have identified mCherry-Rabies labeled ooDSGCs (Figure 2.7). Thus, NF cells receive directionally tuned information directly from the retina.
Figure 2.7. **NF cells in sSC are innervated by ooDSGCs.** A) An AAV helper virus targeted to NF cells (green) was injected to sSC, followed by the Rabies virus (red). Rabies-mediated tracing retrogradely labeled RGCs presynaptic to NF starter cells (green + red merge). B) Representative morphology and stratification of a Rabies-tagged ooDSGC from the retina contralateral to the sSC injection site. C) Representative polar plot demonstrating the DS tuning of an ooDSGC and spikes evoked by drifting gratings moving in 8 directions. D) Direction-selective index (DSI) of retrogradely labeled ooDSGCs (DSI = 0.27±0.03, N = 4 cells). E) Approximately 25% of sampled inputs to NF cells were identified as ooDSGCs.

**SAC ablation causes a loss of retinal DS**

To determine whether the feature representation of motion direction by NF cells in sSC is important to predation, we sought to render these cells non-DS while maintaining the excitatory drive through the circuit (i.e., maintain NF cell light responses but strip them of DS tuning). Previous reports have shown that cells in sSC that encode the direction of motion inherit this property from the direction-selective circuitry in the retina (Shi et al. 2017). We, therefore, sought to render the retina non-DS by ablating the circuitry responsible for this computation.

Retinal direction selectivity is encoded by oDSGCs and ooDSGCs as a result of coordinated inhibition received from starburst amacrine cells (SACs) in response to a null direction of motion. As such, DSGCs spike robustly to a preferred and similar directions of motion but are silenced in response to motion in the opposite direction. We, therefore, crossed ChAT-Cre mice to a transgenic line that conditionally expressing DTR to selectively ablate SACs in mice receiving bilateral intraocular (i.o.) injections of DT.

Immunostaining against ChAT revealed a loss of SACs from these retinas more than 1 week after DT injection (Figure 2.8A-B). Two-photon guided patch clamp recordings of ooDSGCs in
these retinas revealed a reduction in direction selectivity but persistent light responses, suggesting that the postsynaptic of ooDSGCs to NF cells would maintain its excitatory drive but lose its directional tuning (Figure 2.8C-E). Further, we tested the OKR of these mice as a functional readout to determine whether the consequences of the retinal manipulation would be observable at the level of behavior. As expected, mice lacking SACs and the resulting DS tuning lacked an OKR (Figure 2.8F-H).

Abolition of retinal DS during training phase has no effect on hunting performance
These mice were next tested on the hunting assay to determine whether the DS feature representation was necessary for approach and pursuit of prey. These mice were reliably able to approach and pursue crickets at test, with no observed deficits in capture time, conversions of approach to contact, conversions of contact to capture or in their bearing toward the cricket (Figure 2.9).

Figure 2.9. Predation persists following the loss of retinal DS prior to training. A) Experimental schedule. Intraocular DT or PBS injections were performed more than 1 week before the hunting training phase began. Mice injected with DT in this condition lacked retinal DS prior to first exposure to prey. Mice were tested directly once performance plateaued. B) Representative hunting overviews of mice that received intraocular injections of PBS (grey) or DT (orange) pursuing a cricket (black). C1-F1) Cumulative density plots generated for all trials performed, pooled across all mice tested. N = 60 control and N = 52 DT trials. C1) Time from introduction of a cricket to its capture (Control = 29.44±6.57, DT = 30.72±5.15, p>0.05). D1) Latency to detect prey and initiate new approaches (Control = 2.14±0.34, DT = 2.18±0.29, p>0.05). E1) Probability that mice successfully convert approaches into contacts (Control = 0.72±0.03, DT = 0.66±0.04, p>0.05). F1) Probability that mice successfully convert contacts into captures (Control = 0.29±0.04, DT = 0.23±0.05, p>0.05). G1) Probability distribution of cricket azimuth during approaches or contacts (p>0.05). C2-G2) Summary plots for all mice tested. Each data point represents an average of 3-4 trials per condition for each mouse. N = 15 PBS and N = 12 DT mice. C2) Time from introduction of a cricket to its capture (Control = 2.44±8.15, DT = 29.10±6.84, p>0.05). D2) Latency to detect prey and initiate new approaches (Control = 2.14±0.45, DT = 2.09±0.42, p>0.05). E2) Probability that mice successfully convert approaches into contacts (Control = 0.29±0.05, DT = 0.67±0.04, p>0.05). F2) Probability that mice successfully convert contacts into captures (Control = 0.29±0.05, DT = 0.23±0.05, p>0.05). G2) Mean cricket azimuth during approaches or contacts (Control = 29.33±3.89, DT = 35.00±5.13, p>0.05).

49
These results suggest that the DS feature representation is not required for behavior. However, they leave open the possibility that these mice might hunt live prey using a strategy that differs from their sSC-, V1- or AOS-manipulated counterparts. As the manipulations to the brain all employed iDREADDs to reversibly silence the targets of interest, these mice all developed an innate hunting strategy during training that was acutely impaired at test by the application of the CNO i.p. injection. In contrast, the ChAT-DTR mice received DT i.o. injections more than a week before beginning the training phase of the behavior to ensure that SACs would be ablated by test. As a result, the ChAT-DTR mice received DT injections early enough that their retinas were rendered non-DS by the time they began the training phase of the behavior (i.e., their only interactions with crickets occurred in the absence of retinal DS). We, therefore, sought to test for compensatory hunting strategies by manipulating retinal DS on a time scale that approached the resolution of the iDREADDs-based manipulations in the brain.

*SAC ablation follows a stereotyped time course*

Given the effectiveness of the ChAT-DTR manipulation, we sought to convert this manipulation to an acute time scale. We injected ChAT-DTR mice with DT and began monitoring OKR daily following the i.o. injections (Figure 2.10B). Whereas control, PBS-injected mice maintained the reflex stably over time, the DT-injected mice began to show a functional deficit approximately 8 days post-injection. The reflex was completely abolished across all DT-injected mice by 10 days post-injection.

In a separate cohort of mice, we sacrificed mice injected with DT daily and prepared the retinas to quantify the number of ON- and OFF-SACs present in representative fields of view. Like the OKR, the number of SACs present remained relatively stable for at least 8-9 days after DT
injection across both retinas and mice. By day 10, however, both ON and OFF sacs were reliably ablated across mice (Figure 2.10C-E).
Figure 2.10. DT-induced ablation of SACs follows a stereotyped time course. A) Experimental schedule to determine the time course of SAC ablation. OKR was monitored daily after bilateral intraocular injections of DT or PBS to ChAT-DTR mice. In a separate cohort of mice, retinas were prepared daily and stained against ChAT to visualize ON- and OFF-SACs. B) Quantification of OKR as a function of time, post-intraocular injection (p<0.01). N = 8 PBS- and N=8 DT-injected mice. C, D) Representative retinal whole-mount preparations with staining against ChAT to identify ON- (C1-C4) and OFF- (D1-D4) SACs, on 3, 8, 9, and 10 days after DT injection. E) Quantification of surviving SACs, beginning 3 days after DT injection. N = 2-6 retinas from 1-3 mice per day after DT injection, each retina with 4 ON and 4 OFF fields of view averaged. E1) Surviving ON-SACs (p<0.01). E2) Surviving OFF-SACs (p<0.01).
Together, these results indicate that SAC ablation follows a stereotyped time course following DT injection. Moreover, the reliability of these results between mice suggested that the DT injection could be timed with the initiation of the hunting experiment, such that mice could train on the behavior during a period of relatively normal function and tiling and test immediately after the functional and anatomical loss of SACs.

*Predation is unaffected by SAC ablation during the testing phase*

To align the timing of SAC ablation with the plateauing of hunting performance and test, mice were food restricted and began training with crickets in the arena 4 days after bilateral DT i.o. injections (Figure 2.11A). After 4 days of training, the behavioral performance of the mice plateaued. Consistent SAC ablation was achieved between 9 and 10 days after DT injection. Therefore, none of the mice were tested 9 days after the DT injection to minimize variability in the level of SAC ablation between mice. Instead, mice remained food-restricted without access to crickets on day 9 to prevent mice that might have undergone early DT-induced SAC ablation from developing a compensatory hunting strategy. All mice were instead tested 10 days after DT injection and sacrificed shortly after completing the hunting assay to confirm SAC ablation.

Following the acute loss of SACs 10 days after DT injection, DT-injected and control mice performed equivalently (Figure 2.11). These results phenocopied the performance of mice that underwent early DT injections such that SACs would be ablated during the training phase of the behavior. Together, these results exclude a possibility of a compensatory hunting strategy developed in the absence of retinal DS during training and demonstrate that the DS feature representation encoded in sSC and inherited from the retinal circuitry is not required for efficient predation.
2.1. Loss of retinal DS that coincide with test does not prevent efficient hunting. A) Experimental schedule. Mice received intraocular injections of DT or PBS. Behavioral training (Train) began 5 days after injections, during a period of normal SAC function (see Figure 2.10A) and tiling (see Figure 2.10C-E). Test occurred 10 days post-injection to coincide with DT-induced ablation and loss of function. B) Representative hunting overviews of mice injected with PBS (grey) or DT (orange) pursuing a cricket (black). C1-F1) Cumulative density plots generated for all trials performed, pooled across all mice tested. N = 36 control and N = 34 DT trials. C1) Time from introduction of a cricket to its capture (Control = 21.07±2.92, DT = 24.94±2.75, p>0.05). D1) Latency to detect prey and initiate new approaches (Control = 1.24±0.17, DT = 1.63±0.22, p>0.05). E1) Probability that mice successfully convert approaches into contacts (Control = 0.69±0.03, DT = 0.66±0.03, p>0.05). F1) Probability that mice successfully convert contacts into captures (Control = 0.20±0.03, DT = 0.16±0.03, p>0.05). G1) Probability distribution of cricket azimuth during approaches or contacts across all trials (p>0.05). C2-G2) Summary plots for all mice tested. Each data point represents an average of 3-4 trials per condition for each mouse. N = 9 PBS and N = 9 DT mice. C2) Time from introduction of a cricket to its capture (Control = 21.07±4.37, DT = 24.85±3.48, p>0.05). D2) Latency to detect prey and initiate new approaches (Control = 1.24±0.30, DT = 1.58±0.29, p>0.05). E2) Probability that mice successfully convert approaches into contacts (Control = 0.69±0.03, DT = 0.65±0.03, p>0.05). F2) Probability that mice successfully convert contacts into captures (Control = 0.20±0.04, DT = 0.16±0.03, p>0.05). G2) Mean cricket azimuth during approaches or contacts (Control = 28.89±3.51, DT = 30.00±5.46, p>0.05).

2.4 Discussion

Here, we manipulated direction selectivity to determine the pathway specificity with which this information might contribute to predation and tested the necessity of this key stimulus feature for converting visual input to behavioral output. Our experiments manipulating the direction-selective circuits in the brain and retina reveal 6 key findings outlined below.
First, the pharmacogenetic silencing or ablation of a population of direction-selective NF cells in sSC reliably induces behavioral deficits in predation, impairing tracking and pursuit. Second, the contributions of visual pathways that encode the direction of motion to visual behaviors are specific and non-overlapping. Silencing of NF cells in sSC induced deficits only in predation, whereas silencing of the dLGN-V1 and AOS pathways affected only depth perception (i.e., visual cliff) and gaze stabilization (i.e., OKR), respectively.

Third, the NF cells in sSC that contribute to predation are directly innervated by ooDSGCs, demonstrating that the direction-selective tuning of these cells should depend on retinal circuitry, as previously reported (Shi et al. 2017). Fourth, retinal direction selectivity can be eliminated by targeting DT-mediated ablation to SACs. Despite abolishing direction selectivity, SAC ablation maintains excitatory drive through DSGCs, and its effect can be observed behaviorally in the loss of OKR. Fifth, DT-mediated ablation of SACs follows a stereotyped time course that can be leveraged for behavioral experiments, with cell death and loss of function coinciding by 10 days after DT injection. Last, the loss of retinal direction selectivity, whether chronically to affect the training and test phases of predation or acutely to affect only the test phase, does not affect predation.

This pattern of results stands in contrast to the circuitry previously described between the retina and sSC projections. ooDSGCs encoding direction of motion innervate parabigeminal-projecting sSC neurons, which include NF cells (Reinhard et al. 2019; Gale and Murphy 2014). These links connect ooDSGCs to putative circuitry that could be used to generate orienting head and eye movements toward moving stimuli of interest, such as prey. Despite this link and our observation that NF cells perturb the ability of mice to pursue prey smoothly, mice retain the ability to efficiently hunt – including the ability to orient toward prey - in the absence of information about
how their prey moves. Our retinal manipulations in this context are significant because they maintain excitatory drive through the NF cells participating in this behavioral circuit while altering their tuning. Thus, rather than identifying that NF cells participate in the circuit, these experiments help elucidate how NF cells contribute to the computations performed.

The result that the loss of direction selectivity is not sufficient to phenocopy the effect of either silencing or ablating NF cells indicates that the ability of NF cells to multiplex by integrating the inputs of varied RGC types is more important than a single defining feature of their preferred stimulus repertoire. Alternatively, the ability of NF cells to encode other stimulus features consistent with insect prey, such as size or speed, might drive approach initiations and therefore be sufficient to maintain efficient predation absent their directional coding. Thus, the combined inputs from non-direction-selective cell types, which remain to be characterized, coupled with the preserved excitatory drive through NF cells, are sufficient to preserve predation.

Alternatively, although NF cells receive and integrate visual inputs from the retina, their outputs help drive the motor circuitry that performs the act of predation. Thus, the silencing or ablation of NF cells might be sufficient to reduce drive to the motor pathways enough to reduce the reliability of predation. Such unreliability might account for the variability observed between trials of a single manipulated mouse and remains to be tested.

2.5 References


Chapter 3: Contributions of Ipsilaterally Projecting Sustained ON and OFF Alpha Retinal Ganglion Cells to Predation

3.1 Introduction

There are more than 40 unique circuits in the retina that collectively innervate 50+ targets in the brain to collectively guide visual behaviors (Martersteck et al. 2017; Bae et al. 2018; Tran et al. 2019; Baden et al. 2016). The precise wiring of a subset of these circuits – for example, the direction-selective circuit described in Chapter 2 of this dissertation – has been determined. The significance to visual behavior of most retinal circuits, however, remains unknown. Our efforts to understand how specific retinal circuits and cell types contribute to behavior are limited by the availability of tools to target and manipulate cell types of interest reliably and precisely. Many of the genetic strategies currently used to manipulate cell types in the retina target groups of cell types, preventing researchers from drawing specific conclusions about individual cell types.

Recently, mice were shown to rely on 9 retinal ganglion cell types that project ipsilaterally (ipsi-RGCs) to effectively hunt insects, such as crickets (Johnson et al. 2021). This subset of 9 ipsi-RGCs constitutes approximately 2% of RGCs in the retina but occupies approximately 20% of the retina and informs binocular vision (Johnson et al. 2021). This subpopulation of cells therefore represents a specialized group of RGCs that evolutionarily has been thought to improve visual acuity – especially under poor light conditions – by effectively doubling the sampling of available light in the same region of visual space (Heesy 2008).
Whether all 9 of these ipsi-RGCs are necessary to guide efficient predation and how they execute this function could not be inferred from the manipulations performed by Johnson et al. (2021). An electrophysiological characterization of ipsi-RGCs revealed that only a subset (5/9) of contrast-encoding ipsi-RGCs encoded meaningful stimulus information that could signal the movement of prey within the visual field of a mouse (Johnson et al. 2021). This suggested that among all ipsi-RGCs, there might be further specialization or designation of function.

Binocular vision in specialized predators is complemented by the presence of an area centralis (Rapaport and Stone 1984). The area centralis is a small region in the temporal retina, in which RGCs typically have smaller dendritic arbors and receptive fields, while maintaining the precise tiling necessary to completely sample visual space (Rapaport and Stone 1984; Boycott and Wässle 1974; Stone and Fukuda 1974). As a result, convergence from photoreceptors and bipolar cells to RGCs is reduced (Wässle and Boycott 1991) to increase visual acuity. Depending on species, not all RGCs in this retinal space exhibit these properties (Rapaport and Stone 1984; Provis 1979). In monkeys and cats, for example, only cells such as the contrast-encoding X-like and X cells exhibit these properties (Stone and Fukuda 1974; Rapaport and Stone 1984). Given the diversity of cell types in the mouse retina, however, such a cell-type-specific effect might be harder to localize.

For decades, it has been thought that mice lack an area centralis or area of retinal specialization that can support enhanced visual acuity. RGCs appeared to be distributed evenly across the mouse retina and without an apparent size or density gradient (Dräger and Olsen 1981). Recently, (Bleckert et al. 2014) found that among all RGC types, sustained ONα (sONα) and sustained OFFα (sOFFα) RGCs vary in density and dendritic arbor size along a nasal-temporal gradient and are smaller and denser in temporal retina. sONα and sOFFα RGCs are paramorphic
RGC types included within the population of ipsi-RGCs (2/9) found in the mouse ventrotemporal retina (Johnson et al. 2021). Moreover, ipsi-sONα and ipsi-sOFFα reliably encode information about moving prey (2/5). Of all 9 ipsi-RGC types, only ipsi-sONα and ipsi-sOFFα RGCs exhibited smaller dendritic arbors and receptive field sizes than their contralaterally-projecting counterparts elsewhere in the retina (2/9) (Johnson et al. 2021).

Based on these findings, ipsi-sONα and ipsi-sOFFα RGCs appear to share features that are common to the area centralis specialization frequently observed in predators. We hypothesized that ipsi-sONα and ipsi-sOFFα RGCs, specifically, confer many of the visual advantages utilized by predators to hunt and that these 2 cell types would be required to guide predation. Therefore, we sought to characterize the distribution of these cells in the retina, their projections to the brain, and their contributions to predatory behaviors.

### 3.2 Method

**Animals**

The procedures in this study were approved by the Animal Studies Committee of Washington University School of Medicine (Protocol #20200055) and performed in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

SERT-Cre (Gong et al. 2007) and SERT-Flp (Ren et al. 2019) mice were used to target genetic manipulations to the population of ipsi-RGCs. For ipsi-RGC ablation experiments, SERT-Cre mice were crossed to a transgenic mouse line in which the diphtheria toxin receptor (DTR) is expressed in a Cre-dependent manner (DTR mice; (Buch et al. 2005). To target a subset of ipsi-αRGCs, SERT-Flp mice were crossed to KCNG4-Cre mice (Duan et al. 2014) to leverage the
intersection of these transgenic lines (SERT-KCNG4 mice). To fluorescently label ipsi-αRGCs for 2-photon guided patch clamp experiments, SERT-KCNG4 mice were crossed to Ai80 mice, in which the YFP is conjugated to channelrhodopsin CatCh and expressed only in cells expressing both Cre and Flp (Daigle et al. 2018). To silence ipsi-αRGCs for predation experiments, the SERT-KCNG4 mice were crossed mice expressing the tetanus toxin under the control of Cre and Flp (Tox mice) (Kim et al. 2009). GRP-Cre mice were used to manipulate narrow-field (NF) cells in superficial superior colliculus (sSC; (Gerfen, Paletzki, and Heintz 2013).

**Surgical Procedures**

For all surgical procedures, mice were anesthetized by intraperitoneal (i.p.) injection of ketamine/xylazine cocktail (0.1 mg/10 g). Meloxicam (5 mg/kg) was administered post-operatively by subcutaneous injection at 0, 24 and 48 hours. The scalp was sanitized with betadine solution and 70% ethanol before incisions were made to expose the cranial bone for craniotomy.

**Rabies-mediated retrograde tracing**

The sSC of GRP-Cre was injected unilaterally via a large craniotomy over sSC with a Cre-dependent helper AAV encoding an envelope protein, GFP, and glycoprotein (G protein), as described in Chapter 2 of this dissertation. Following virus expression, a G-deleted Rabies virus tagged with mCherry was injected to the same stereotactic coordinates to retrogradely label synaptic inputs to GRP+ NF cells in sSC.

**Retrograde tracing of retinal inputs**
A nanoinjector (Nanoject III, Drummond Scientific) fitted with a pulled glass pipette was used to deliver 100-200 nL of Cholera toxin, subunit B conjugated to AlexaFluor 488 or 568 (CTB-488 or CTB-568) to dorsolateral geniculate nucleus (dLGN). Coordinates were determined relative to Bregma. Surgical sites healed and stables were removed 7-10 days after injections. These injections retrogradely labeled dLGN-projecting RGCs.

**Anterograde tracing of RGC terminals**

CTB-488 was injected intravitreally into one eye using a nanoinjector fitted with a pulled glass pipette (Nanoject II, Drummond Scientific) to label terminals of RGCs projecting ipsilaterally and contralaterally.

**Ablation of ipsilaterally projecting RGCs**

Diphtheria toxin (DT; 1.6 ng in 2 µL PBS) was delivered bilaterally to SERT-DTR mice by intraocular injection to selectively ablate ipsilaterally projecting RGCs, using a protocol modified from Hillier et al. (2017). A subset of littermate SERT-DTR mice were injected using the same protocol with PBS as control.

**Behavioral testing**

**Predation**

SERT-DTR and SERT-KCNG4-Tox mice and their respective controls were trained to hunt crickets in an arena, as described in Chapter 2 of this dissertation and similar to protocols previously implemented by (Johnson et al. 2021; Hoy et al. 2016; Hoy, Bishop, and Niell 2019). The mice were trained in the arena 4-5 days until their performance plateaued and then were tested the following day with no additional manipulation.
**Tissue preparation**

Mice were deeply anesthetized with CO₂ and killed by cervical dislocation. As necessary, brains were harvested and fixed in 4% paraformaldehyde (PFA) overnight before being transferred to 1X PBS prior to slice collection for staining. For immunohistochemistry experiments, eyes were removed and transferred to oxygenated mACSF containing (in mM): 119 NaCl, 2.5 KCl, 1 NaH₂PO₄, 2.5 CaCl₂, 1.3 MgCl₂, 20 HEPES and 11 glucose (pH adjusted to 7.37 using NaOH). Retinas were isolated, mounted on filter paper (Millipore), and fixed with 4% PFA for 30 minutes.

For electrophysiology experiments, mice were dark-adapted for more than 1.5 hours before being deeply anesthetized with CO₂ and killed by cervical dislocation. The eyes were removed under dim red light and transferred to mACSF equilibrated with 95:5, O₂/CO₂, containing (in mM): 125 NaCl, 2.5 KCl, 1 MgCl₂, 1.25 NaH₂PO₄, 2 CaCl₂, 20 glucose, 26 NaHCO₃ and 0.5 L-glutamine. The retinas were isolated under infrared illumination (>900 nm) and were flat-mounted, ganglion cell side-up on transparent glass membrane discs (13 mm, Whatman Anodisc).

**Immunohistochemistry**

For the brains of Ai80-SERT-KCNG4 mice, vibratome slices were prepared (thickness, 100 µm) to cover all 50+ retinorecipient brain areas. For brains of GRP-Cre mice following Rabies tracing experiments and SERT-KCNG4-Tox mice and littermate controls that received intravitreal CTB injections, vibratome slices were prepared to cover SC. Slices were blocked in 10% normal donkey serum (NDS) in PBS for 2 hours. Slices were then incubated for 2 days in primary
antibody at 4°C. Slices were washed 3 times in PBS for 30 minutes and incubated in secondary antibody for 2 hours.

Flat-mounted retinas were cryoprotected (1 hour in 10% sucrose in PBS, 1 hour in 20% sucrose in PBS, overnight at 4°C in 30% sucrose in PBS) and then frozen and thawed 3 times with liquid N2. Retinas were blocked in 10% NDS in PBS for 2 hours and then incubated in primary antibody for 5 days at 4°C. Retinas were washed 3 times with PBS for 1 hour and then incubated in secondary antibody for 2 days at 4°C.

The following primary antibodies were used in this study: rabbit anti-dsRed (1:1000), rabbit anti-GFP (1:1000), rabbit anti-synaptobrevin/VAMP2, goat anti-sPP1 (1:40) and mouse anti-SMI32 (1:1000). Secondary antibodies were Alexa 488 and 568 conjugates. Nuclei were labeled with DAPI to determine retina or brain anatomy.

**Mosaic analysis**

The positions of ipsi-sONα and ipsi-sOFFα RGCs of Ai80-SERT-KCNG4 retinas were mapped; dendritic stratification within the inner plexiform layer of the retina was used to differentiate between ON and OFF cells. The relative numbers of ipsi-sONα and ipsi-sOFFα RGCs present in the ganglion cell layer (GCL) and inner nuclear layer (INL) were calculated. The soma positions of ipsi-sONα or ipsi-sOFFα RGCs present in the GCL and INL were collapsed to yield independent soma position maps for ipsi-sONα and ipsi-sOFFα RGCs. In each map, a reference cell was selected, and the relative density (normalized # cells/area) of neighboring cells in annuli extending from the reference cell in 15 µm intervals was calculated to compute the density recovery profiles (Rodieck 1991).
**Electrophysiology**

Dark-adapted retinas were prepared, as described, and superfused (~7 mL/min) with warm (30-33°C) mACSF$_{NaHCO_3}$. Fluorescently labeled RGCs were targeted under 2-photon guidance (excitation wavelength: 940 nm) in retinas from Ai80-SERT-KCNG4 mice or following Rabies retrograde tracing experiments. Current-clamp recordings were performed with intracellular solution that was pH-adjusted to 7.2 with KOH and that contained (in mM): 125 K-gluconate, 10 NaCl, 1 MgCl$_2$, 10 EGTA, 5 HEPES, 5 ATP-Na$_2$ and GTP-Na. Borosilicate glass pipettes used had resistances between 3-6 MΩ. Signals were amplified with a Multiclamp 700B amplifier (Molecular Devices), filtered at 3 kHz (8-pol Bessel low-pass), and sampled at 10 kHz (Digidata 1550, Molecular Devices).

*Visual stimulation*

All visual stimuli were written using the Cogent Graphics toolbox (John Romaya, Laboratory of Neurobiology at the Wellcome Department of Imaging Neuroscience, University College London) in MATLAB (The MathWorks). Stimuli were presented with a UV E4500 MKII PLUS II projector illuminated by a 385-nm light-emitting diode (LED, EKB Technologies). Stimuli were focused onto the photoreceptors underlying the soma of the recorded cell through the substage condenser on an upright 2-photon microscope (Scientifica). A background luminance of 3,000 rhodopsin isomerizations/rod/s (R*) was used for all visual stimuli.

Different size circles (0, 100, 200, 300, 600, 1200 µm) were presented in pseudo-random order at 100% Michelson contrast to determine the receptive field size and ON/OFF polarity of the recorded cells. A full-field chirp stimulus (Baden et al. 2016) was presented to assess the temporal frequency and contrast responsiveness of the recorded cells.
Quantification and statistical analysis

No statistical methods were used to predetermine sample sizes. P values were calculated using Mann-Whitney U tests or Kruskal-Wallis tests, where appropriate. Population data are reported as mean ± SEM, and N represents the number of trials, animals or cells analyzed.

3.3 Results

An intersectional genetic strategy offers a solution to target a subpopulation of ipsi-RGCs

Given the morphologic, genetic, and functional diversity of cell types in the retina, identifying individual or small combinations of marker genes that can be used to manipulate a particular RGC type selectively is challenging (Sanes and Masland 2015; Martersteck et al. 2017). The αRGCs have successfully been characterized using the KCNG4-Cre transgenic line, which labels sONα, sOFFα, and transient OFFα (tOFFα) RGCs (Duan et al. 2015). Ipsi-RGCs, on the other hand, express the serotonin transporter (SERT) during development (Koch et al. 2011). We previously used a SERT-Cre transgenic line (Gong et al. 2007) to label ipsi-RGCs with high fidelity and characterize the 9 ipsi-RGC types that support binocular vision, which include the sONα and sOFFα RGCs (Johnson et al. 2021).

SERT-Cre and KCNG4-Cre transgenic lines intersect only in their targeting of sONα and sOFFα RGCs. Given a method to target both SERT+ and KCNG4+ cells in the same retinas, a SERT transgenic line would therefore isolate the ipsilaterally projecting subset of KCNG4+ αRGCs (i.e., ipsi-sONα and ipsi-sOFFα RGCs, collectively referred to as ipsi-αRGCs). To this end, we crossed SERT-Flp mice to KCNG4-Cre mice to test whether we could reliably target ipsi-αRGCs using intersectional reporters (Daigle et al. 2018).
The intersection of SERT-Flp and KCNG4-Cre labeled a population of approximately 260 RGCs in the ventrotemporal retina (Figure 3.1) of mice expressing the Ai80 intersectional reporter (Daigle et al. 2018). The location of these cells suggested ipsilateral projections. We injected CTB into one hemisphere of dLGN to retrogradely label dLGN-projecting RGCs and quantified the number of cells expressing Ai80 and CTB in ipsilateral and contralateral retinas relative to the dLGN injection site. This analysis revealed that nearly all the Ai80-expressing cells in the ipsilateral retina were co-labeled with CTB, whereas few if any, Ai80-expressing cells in the contralateral retina also expressed CTB (Figure 3.2). Co-expression of Ai80 and CTB was restricted to the ipsilateral retina, indicating that this intersectional genetic approach specifically labels a subset of ipsi-RGCs.

Figure 3.1. Genetic intersection of SERT-Flp and KCNG4-Cre labels a subpopulation of RGCs in ventrotemporal retina. A whole-retina micrograph of RGCs labeled by the Ai80 intersectional reporter. The highest density of labeled cells (~260 cells total) occurs in the ventrotemporal retina.
Figure 3.2. SERT-KCNG4 RGCs project ipsilaterally. CTB was injected unilaterally to dLGN to distinguish between ipsilaterally and contralaterally RGCs. The degree of overlap between SERT-KCNG4 RGCs (green) and CTB-labeled RGCs in ipsilateral and contralateral retinas was determined. A) RGCs in the ipsilateral retina that were labeled by the intersection of SERT-KCNG4 and by CTB overlapped extensively. In contrast, B) the labeling of intersection of SERT-KCNG4 and of CTB did not colocalize in the contralateral retina. C) The probabilities of SERT-KCNG4 RGCs being co-labeled by CTB were calculated in the ipsilateral and contralateral retinas. Co-labeling was restricted to ipsilaterally projecting RGCs.

Intersection of SERT-Flp and KCNG4-Cre specifically targets ipsi-αRGCs

We implemented a combination of immunohistochemistry and 2-photon guided patch-clamp recordings to characterize the cell type identities of the ipsi-RGCs isolated in Ai80-SERT-KCNG4 mice.

As a population, αRGCs can be labeled with antibodies against neurofilaments and osteopontin (Duan et al. 2015; Bleckert et al. 2014; Krieger et al. 2017; Tan et al. 2022). Staining for the non-phosphorylated neurofilament heavy chain using a SMI-32 antibody labels sONα and tOFFα RGCs (Bleckert et al. 2014), whereas staining for osteopontin with the SPP-1 antibody labels all αRGCs, including the sOFFα RGC (Krieger et al. 2017). The ventrotemporal cells labeled in Ai80-SERT-KCNG4 retinas expressed SMI-32 or osteopontin markers, confirming their identity as αRGCs (Figure 3.3).
Figure 3.3. SERT-KCNG4 RGCs are αRGCs. Retinas with SERT-KCNG4 RGCs genetically labeled (green) were stained against SMI-32 and SPP-1 (magenta) to identify putative αRGCs. A) SERT-KCNG4 RGCs were co-labeled with SMI-32, identifying subsets of SERT-KCNG4 RGCs as sONα or tOFFα RGCs. B) SERT-KCNG4 RGCs were co-labeled with SPP-1, identifying subsets of SERT-KCNG4 RGCs as any αRGC subtype. C) The probabilities of SERT-KCNG4 RGCs being co-labeled with either SMI-32 or SPP-1 were calculated. Nearly all SERT-KCNG4 RGCs were labeled by either SMI-32 or SPP-1, indicating that SERT-KCNG4 RGCs are αRGCs.

Immunostaining alone was insufficient to determine which of the αRGC subtypes were included in this subset of ipsi-RGCs. Therefore, whole-cell patch-clamp recordings were performed to differentiate between the transient and sustained types of ONα and OFFα RGCs, respectively. We measured the responses of these cells to varying size spots and a full-field chirp stimulus while simultaneously filling the RGCs with fluorescent dye to visualize dendritic morphology. All recorded cells displayed sustained responses to light onset or offset, consistent with sONα and sOFFα RGCs, respectively (Figure 3.4).
Figure 3.4. Electrophysiological profiling identifies SERT-KCNG4 RGCs as sONα and sOFFα RGCs. Whole-cell patch-clamp recordings were performed on RGCs labeled by the intersection of SERT-KCNG4. Recorded cells were filled with fluorescent dye and imaged. Top: Representative z-stack projection of an ipsi-sONα RGC. A2) Maximal firing rates of all ipsi-sONα RGCs recorded in response to ON and OFF contrast spots of varying sizes. A3) Representative firing rate of an ipsi-sONα RGC in response to a full-field chirp stimulus to determine cellular contrast and temporal frequency preferences. B1-3) Data presented as in A for ipsi-sOFFα RGCs.

Ipsi-αRGCs fully sample binocular visual space

The area centralis mediates high acuity vision based on the higher density and smaller receptive fields of RGCs present in this area compared to the rest of the retina (Rapaport and Stone 1984; Boycott and Wässle 1974; Stone and Fukuda 1974). Previous reports have shown that sONα and sOFFα RGCs are smaller in the ventrotemporal retina than their counterparts elsewhere in the retina (Bleckert et al. 2014; Johnson et al. 2021). We, therefore, tested whether the ipsi-sONα and ipsi-sOFFα RGCs labeled by the intersection of Ai80-SERT-KCNG4 formed the cellular mosaic necessary to completely sample the binocular visual field. We mapped the position of ipsi-sONα and ipsi-sOFFα somas within a field of view (Figure 3.5A) and calculated the density of neighboring ipsi-sONα or ipsi-sOFFα RGCs relative to reference cells of each type. A substantial proportion of ipsi-sOFFα RGCs were displaced from the ganglion cell layer (GCL) to the inner nuclear layer (INL) (Figure 3.5B) and therefore conventional RGCs and displaced
RGCs were mapped together. Both ipsi-sONα and ipsi-sOFFα RGCs featured an area of exclusion, indicated by decreased cellular density immediately surrounding reference cells (Figure 3.5C). Consistent with findings from (Bleckert et al. 2014) that compared the spatial distribution of all sONα RGCs in nasal and temporal regions of the retina (i.e., without regard for ipsilateral vs. contralateral projections), the peak density for ipsi-sONα and ipsi-sOFFα RGCs was reached by an inter-soma distance of ~100 µm. These results suggest that the ipsi-sONα and ipsi-sOFFα RGCs each form an independent cellular mosaic that can sample binocular visual space with high acuity.

Figure 3.5. Ipsi-αRGC projections to the brain

A recent study anatomically identified the targets in the brain that receive direct innervation from ipsilaterally and contralaterally projecting RGCs (Martersteck et al. 2017). This study revealed that 34 nuclei receive input from contralaterally projecting RGCs, and of these nuclei, 25 also receive input from ipsi-RGCs (Martersteck et al. 2017). The 9 ipsi-RGC types represent classes
of RGCs that participate in either image- or non-image forming vision (Johnson et al. 2021) and thus inform discrete visual pathways in the brain. Therefore, we did not expect all ipsi-RGC subtypes to participate in all visual pathways that are innervated broadly by ipsi-RGCs.

To determine where and how high-acuity binocular information carried by ipsi-sONα and ipsi-sOFFα RGCs might be integrated in the brain, we mapped their terminals to the subcortical nuclei that they innervated. Consistent with previous reports, ipsi-αRGC terminals were detected in the core region of dorsolateral geniculate nucleus (dLGN; Figure 3.6A), superficial layers of superior colliculus (sSC; Figure 3.6B), and the olivary pretectal nucleus (OPN; Figure 3.6D) (Johnson et al. 2021; Martersteck et al. 2017; Morin and Studholme 2014; Young and Lund 1998). We also detected a weak projection to the nucleus of the optic tract (NOT; Figure 3.6C), inconsistent with findings from (Martersteck et al. 2017), which describes only a contralateral projection to NOT. Given the proximity of NOT and OPN, however, the weak NOT labeling might be mis-attributed labeling from OPT and remains to be confirmed.

![Figure 3.6: Ipsi-αRGC projections to subcortical visual centers.](image)

Figure 3.6. Ipsi-αRGC projections to subcortical visual centers. Ipsi-αRGC terminals were detected in A) the core region of the dorsolateral geniculate nucleus (dLGN), B) superficial superior colliculus (sSC), C) the nucleus of the optic tract (NOT), and D) the olivary pretectal nucleus (OPN).

Given the observed projection pattern, it is most likely that high-acuity binocular information is used by image-forming visual centers, such as dLGN or sSC, to guide visual behaviors. While this population of cells might aid in higher-order visual processing, such as stereopsis to support depth perception through the dLGN-V1 visual pathway, ipsi-RGCs are known to be necessary
for guiding predatory behaviors (Johnson et al. 2021). Visual circuits in sSC have been previously implicated in hunting deficits (Hoy, Bishop, and Niell 2019; Chapter 2, Figure 2.1), suggesting a behavioral pathway to which ipsi-αRGCs might therefore provide a crucial input. In contrast, our data suggest that neither the dLGN-V1 pathway nor the accessory optic system, which includes NOT, are required for normal predatory behaviors (Chapter 2, Figures 2.4 and 2.5). While the role of OPN in predation remains to be explored, this nucleus has primarily been implicated in the pupillary light reflex (PLR) in mice, which is mediated by intrinsically photosensitive ipRGCs, though it might support processing of small, moving objects (Levine and Schwartz 2020). Notably, the sONα RGC expresses a low level of the melanopsin photopigment and shares the same molecular identity as the M4 ipRGC (Sonoda, Okabe, and Schmidt 2020). Thus, synaptic input to OPN from the putative ipsi-sONα RGCs might contribute primarily to the ipRGC-driven PLR, rather than predation. We do not, however, exclude a potential role of OPN in predation.

*Ipsi-αRGCs have a crucial role in behavior*

To test whether ipsi-αRGCs guide predatory behaviors, we crossed the SERT-KCNG4 mice to a transgenic line that expresses tetanus toxin (Tox), given the intersection of SERT-Flp and KCNG4-Cre (Kim et al. 2009). Tox cleaves the synaptic-vesicle-associated membrane protein, VAMP2/synaptobrevin2, and should thus suppress neurotransmitter release from ipsi-αRGC terminals in sSC, dLGN, OPN, and NOT.

As sSC is the most likely postsynaptic target to use inputs from ipsi-αRGCs to guide predation, we confirmed that Tox expression in ipsi-αRGCs effectively reduced VAMP2/synaptobrevin2 expression in ipsi-RGC terminals in sSC. We injected the anterograde tracer CTB-488
intravitreally into a single eye to label all ipsilaterally and contralaterally projecting axon terminals. We then stained axon terminals in ipsilateral and contralateral sSC for VAMP2. As expected, VAMP2 expression colocalized with dense innervation from the contralateral retina but demonstrated little colocalization with the sparse terminals from the ipsilateral retina (Figure 3.7). These results suggest that VAMP2/synaptobrevin2 was cleaved selectively from ipsi-αRGC terminals, putatively silencing the output of ipsi-αRGCs.

![Image](image_url)

**Figure 3.7. Tox expression cleaves VAMP2 from ipsi-αRGC terminals.** CTB (green) was injected to one eye to label all RGC terminals in ipsilateral and contralateral sSC. VAMP2 staining was not detected in terminals projecting ipsilaterally (i.e., putative ipsi-αRGC terminals), but was present in terminals of contralaterally projecting RGCs from the same retina.

We then tested for a functional contribution of ipsi-αRGCs to predation by evaluating the hunting performance of SERT-KCNG4-Tox mice and Cre-, Flp- or Tox- mice on the same genetic background (Figure 3.8). Under the same testing conditions and relative to controls, SERT-KCNG4-Tox mice presented with deficits in the behavior that spanned capture time (Figure 3.8C), the interval between novel approaches (Figure 3.8D), the probabilities of
converting approaches to contacts (Figure 3.8E) or of converting contacts to captures (Figure 3.8F) and the azimuth of the cricket during approach (Figure 3.8G). Notably, the magnitude and direction of the phenotypes observed following the silencing of the ipsi-αRGCs recapitulate those observed after the ablation of all ipsi-RGCs (Figure 3.9), suggesting that the subpopulation of ipsi-αRGCs likely have a dominant role in guiding predation.

Ipsi-αRGCs innervate narrow-field (NF) cells in sSC

Together, the data presented suggest that ipsi-αRGCs might function as a cell-type specific area centralis in mice, indicated by 1) their reduced receptive field size and increased density to sample visual space with higher acuity and 2) their contributions to binocular vision (Rapaport and Stone 1984). In specialized predators, such as primates or cats, directed and stabilizing eye
or head movements shift gaze to keep stimuli of interest focused on the area centralis, given its high visual acuity (Rapaport and Stone 1984). Recent studies have shown that mice rely on the coupling between eye and head movements to smoothly maintain the position of their prey within their binocular visual field (Johnson et al. 2021; Michail, Abe, and Niell 2020). We, therefore, were interested in which subcortical visual circuits might rely on high-acuity input from ipsi-αRGCs.

Ipsi-αRGCs innervate sSC at depths consistent with the sSC sampling space of wide-field (WF) and narrow-field (NF) cells that have previously been implicated in the detection and pursuit of prey, respectively (Hoy, Bishop, and Niell 2019; Gale and Murphy 2014; Cang et al. 2018). Silencing NF cells disrupts several aspects of predation (Chapter 2, Figure 2.1). Although these cells are characteristically direction-selective, our results indicate that the direction selectivity of NF cells is not required for mice to pursue prey efficiently. Instead, we noted that the phenotypes induced by NF cell silencing are largely shared by both the ablation of all ipsi-RGCs and the silencing of ipsi-αRGCs (Figure 3.9). We therefore tested whether ipsi-αRGCs and NF cells participate in the same visual pathway to convert retinal input to behavioral output by testing whether NF cells receive direct input from ipsi-αRGCs.
**Figure 3.9. Behavioral phenotypes are recapitulated between three visual system manipulations.** Ipsi-αRGC silencing (Tox, blue), ipsi-RGC ablation (DT, purple), and NF cell silencing in sSC (CNO, magenta) each induce deficits in time from introduction of a cricket to its capture, in addition to other behavioral metrics reported in this dissertation and in (Johnson et al. 2021). Ipsi-αRGC and NF-CNO data were reproduced from Figure 3.8 and Chapter 2 of this dissertation, respectively, for illustrative purposes. All control groups (i.e., for ipsi-αRGC Tox, ipsi-RGC DTR, and NF-CNO) were combined. A) Cumulative density plot generated for all trials performed, pooled across all mice tested. N = 140 control, N = 27 ipsi-αRGC Tox, N = 20 ipsi-RGC DT, and N = 86 NF-CNO trials. Time from introduction of a cricket to its capture (Control: 16.99±1.79, Tox = 29.96±3.21, DT = 50.75±11.83, CNO = 44.20±5.62, p<0.001). B) Summary plots for all mice tested. Each data point represents the average computed for 1-6 trials for each mouse. N = 35 control, N = 7 ipsi-αRGC Tox mice, N = 4 ipsi-RGC DTR mice, N = 21 NF-CNO mice. Time from introduction of a cricket to its capture (Control = 15.04±1.87, Tox = 30.35±3.09, DT = 50.75±9.46, CNO = 45.60±9.69, p<0.0001).

We performed retrograde Rabies tracing experiments in sSC to label the subset of RGCs that directly innervate NF cells (Figure 3.10A). We then performed 2-photon guided patch-clamp recordings of mCherry-Rabies labeled cells in the ipsilateral retina, relative to the stereotactic brain injections (Figures 3.10B and C). Characterization of these cells revealed that NF cells receive partial input from ipsi-sONα and ipsi-sOFFα RGCs that signal the binocular visual field (Figure 3.10D).
Figure 3.10. Ipsi-sONα RGCs directly innervate NF cells in sSC. A) An AAV helper virus targeted to NF cells (green) was injected to sSC, followed by the Rabies virus (red). Rabies-mediated tracing retrogradely labeled RGCs presynaptic to NF starter cells (green + red merge). B) Representative morphology and stratification of a Rabies-tagged sONα RGC from the retinal ipsilateral to the sSC injection site. C) Maximal firing rates of recorded ipsi-sONα RGCs in response to bright spots of increasing diameter. Responses were characteristically sustained and with limited surround inhibition. D) Approximately 50% of the recorded Rabies-tagged ipsi-RGCs were identified as αRGCs.

Previous electrophysiological characterization of the RGC types that project ipsilaterally and comprise the binocular visual field indicated that only 5 of the 9 ipsi-RGC types were suitable to reliably encode stimuli approximating prey, including ipsi-sONα and ipsi-sOFFα RGCs. Given that ipsi-αRGCs can reliably encode prey and directly innervate NF cells and that silencing either ipsi-αRGCs or NF cells results in the same behavioral phenotype, we hypothesize that ipsi-αRGCs and NF cells participate in a single visual circuit responsible for guiding the efficient pursuit of prey. In this putative circuit, NF cells multiplex high-acuity inputs from ipsi-αRGCs and other contralaterally projecting RGC types, such as direction-selective ganglion cells, to convert visual information about the movement of prey to the motor outputs necessary to move the head and eyes and keep prey focused on the area centralis region of the binocular visual field.

3.4 Discussion

Here, we implemented an intersectional genetic approach to test whether a subpopulation of ipsi-RGCs that might function as an area centralis by encoding prey-like features of visual stimuli with high acuity in the binocular visual field is required for efficient predation. The results of our
experiments indicate 4 key findings and suggest a novel pathway by which binocular vision might require high visual acuity to drive predation.

First, our results indicate that the intersection of SERT-Flp and KCNG4-Cre transgenics reliably isolates a population of ipsilaterally projecting sONα and sOFFα RGCs, allowing for their genetic manipulation. Second, our anterograde tracing of ipsi-αRGC projections to the brain indicate that they innervate few subcortical targets, including sSC, which has previously been implicated in predatory behaviors (Hoy, Bishop, and Niell 2019).

Third, ipsi-αRGCs are required for efficient predation, as silencing this small population of ~260 cells in each retina induces deficits in the pursuit and capture of prey. Notably, the phenotype induced by the silencing of ipsi-αRGCs is strikingly similar to those induced either by the ablation of all ipsi-RGCs or by the silencing of NF cells in sSC (Johnson et al. 2021; Hoy, Bishop, and Niell 2019). Last, the similarity of these phenotypes prompted us to identify the direct, ipsilateral retinal inputs to NF cells in sSC, which revealed that ipsi-αRGCs directly innervate NF cells (Reinhard et al. 2019; Gale and Murphy 2014).

The projections of ipsi-αRGCs to sSC and the retrograde labeling of ipsi-αRGCs from NF cells in sSC together suggest a retinocollicular pathway by which ipsi-αRGCs initiate and guide hunting. The consistency in both direction and magnitude of the phenotypes observed following ipsi-RGC ablation, NF cell silencing, and ipsi-αRGC silencing suggests that the excitatory drive of a small subpopulation of RGCs (260 RGCs per retina; <1% of RGCs) might be sufficient to account for a striking behavioral phenotype previously observed only by coarser manipulations to a visual pathway (Hoy et al. 2016; Hoy, Bishop, and Niell 2019; Johnson et al. 2021).
The influence that ipsi-αRGCs have on this visual behavior might be explained by their putative contribution to visual acuity. Ipsi-αRGCs have smaller dendritic arbors and receptive fields in the ventrotemporal retina (Bleckert et al. 2014; Johnson et al. 2021), yet they form independent mosaics that fully sample the binocular visual field. As a result, the binocular region of visual space is over-sampled by the retina, and these inputs might therefore be preferentially weighted by their postsynaptic partners (i.e., NF cells), though the relative weight of ipsi-αRGC inputs to postsynaptic targets, including NF cells, remains to be tested.

The projection of ipsi-αRGCs to NF cells further links ipsi-αRGCs to the pre-motor deep layers of SC and the parabigeminal nucleus that are involved in generating the motor outputs necessary to direct the head and gaze toward stimuli of interest (Cang et al. 2018; Reinhard et al. 2019; Ma et al. 2013; Sahibzada, Dean, and Redgrave 1986; Wurtz and Albano 1980). The morphological and physiological properties of NF cells might further present a pathway through which the visual acuity achieved by ipsi-αRGCs in the retina can be maintained for active pursuit of prey, in contrast to a separate, lower acuity pathway – likely driven primarily by monocular inputs – that is sufficient for detection of prey (Reinhard et al. 2019; Hoy, Bishop, and Niell 2019; Heesy 2008). This possibility will be discussed in further detail in Chapter 5 of this dissertation.

Finally, although the results presented in this study together demonstrate a clear contribution of a small number of ipsi-αRGCs to predation, they do not exclude a role of the other 7/9 ipsi-RGC types that constitute the binocular visual field that is necessary for efficient predation. These results also do not exclude a role of other RGC types that might signal prey in the periphery (i.e., for detection) to focus the binocular visual field on a stimulus of interest during the pursuit of prey.
3.5 References


Chapter 4: Conclusions and Future

Directions

4.1 Conclusions

This dissertation explored the functional contributions of 2 retinocollicular pathways that encode either 1) motion direction or 2) the binocular visual field to predation.

The study reported in Chapter 2 demonstrates that motion direction – as encoded in the retina or in the brain – is not required for mice to efficiently pursue dynamically moving prey. This result was unexpected, as we observed that silencing the characteristically direction-selective (DS) narrow-field (NF) cells in superficial superior colliculus (sSC) induces striking deficits in the ability of mice to pursue and capture prey. These experiments are among the first to test for a role of a key cellular feature in behavior, as we rendered NF cells non-DS but otherwise maintained excitatory drive through them.

The study reported in Chapter 3 shows that the ipsilaterally projecting subset of sustained ONα (sONα) and OFFα (sOFFα) retinal ganglion cells (RGCs) in the ventrotemporal retina (ipsi-αRGCs) have characteristics consistent with an area centralis. The area centralis is a region of temporal retina that contributes to the binocular visual field and enables high acuity vision via densely packed RGCs with small dendritic arbors and receptive fields (Rapaport and Stone 1984; Boycott and Wässle 1974; Stone and Fukuda 1974). As previously reported, ipsi-αRGCs inform the binocular visual field and are smaller and more densely packed than their counterparts elsewhere in the retina (Bleckert et al. 2014; Johnson et al. 2021). Our results indicate that both
ipsi-αRGCs are densely packed and form independent mosaics that can encode the binocular visual field with high visual acuity. These properties indicate that ipsi-αRGCs can function as an area centralis in mice. Animals with more developed visual systems than mice perform directed and stabilizing eye or head movements to maintain stimuli of interest focused on the area centralis (Rapaport and Stone 1984; Ito and Feldheim 2018). We show that ipsi-αRGCs are required to guide predation. This suggests that mice might also perform eye and head movements to keep their prey centered on an area centralis-like region of ventrotemporal retina.

We find that the retinocollricular pathways investigated in Chapters 2 and 3 of this dissertation converge onto the NF cells in sSC. While the DS tuning of NF cells is not required for predation, how converging inputs are integrated by NF cells to drive behavior remains an open question, as does the functional significance of direction selective signaling.

### 4.2 Future Directions

*Function of direction selectivity in behavior*

The DS circuitry of the retina innervates 3 visual pathways. Yet, only the functional role of ON-DS RGCs (oDSGCs) in generating the compensatory optokinetic reflex via the accessory optic system (AOS) has been successfully characterized. Inputs from ON-OFF DS RGCs (ooDSGCs) are largely excluded from the AOS (Dhande et al. 2013), suggesting that ooDSGCs have a limited functional role in generating the optokinetic reflex. Likewise, the results presented in Chapter 2 of this dissertation indicate that inputs from ooDSGCs to either the dorsolateral geniculate nucleus (dLGN) or sSC are not required for predation. However, this combination of negative results does not exclude a potential role of ooDSGCs in generating gaze-stabilizing and directed eye movements, given the sSC circuitry to which they are wired (Reinhard et al. 2019).
To date, genetic tools that would permit the independent manipulation of oDSGCs and ooDSGCs do not exist. As a result, manipulations to DS circuitry must either 1) render both oDSGCs and ooDSGCs non-DS (i.e., SAC ablation) or 2) inactivate visual pathways that encode DS information. These approaches can be combined, as in Chapter 2 of this dissertation, to deductively isolate the contributions of oDSGCs and ooDSGCs to visual behaviors. However, this combinatorial approach makes it challenging to identify crosstalk between the oDSGC- and ooDSGC-mediated pathways.

DS responses can be detected in layers 2/3 of primary visual cortex (V1) in the absence of retinal DS coding, indicating that the dLGN-V1 pathway can compute motion direction de novo, given non-DS inputs (Hillier et al. 2017). However, recently, translation- and rotation-selective neurons that encode optic flow and are dependent on horizontal retinal DS have been detected in V1 and other higher visual association areas (Rasmussen et al. 2021). Further, afferent projections from visual cortices to AOS nuclei have been reported in some species (Giolli, Blanks, and Lui 2006; Liu, Huberman, and Scanziani 2016). This link might therefore substantiate a mechanism by which ooDSGCs can encode optic flow and utilize existing oDSGC circuitry to aid the generation of compensatory eye movements. If existent, this capability might specifically preserve horizontal optic flow in response to forward motion, as gaze-stabilizing eye movements are abolished when vertical motion-preferring oDSGCs are excluded from the medial terminal nucleus (MTN) (Sun et al. 2015). This hypothesis will remain difficult to test until further tools are developed that can target ooDSGCs independent of oDSGCs.

In addition to the dLGN-V1 pathway, ooDSGCs innervate sSC such that the tuning of sSC is dependent on retinal DS coding (Shi et al. 2017). Our results agree with other converging evidence (Gale and Murphy 2014; Reinhard et al. 2019) to indicate that ooDSGCs directly
innervate NF cells. NF cells project to the parabigeminal nucleus and deep layers of SC (Gale and Murphy 2014; Cang et al. 2018), both of which are involved in generating the motor outputs necessary to initiate directed head and eye movements toward stimuli of interest (Ma et al. 2013; Sahibzada, Dean, and Redgrave 1986). While DS encoding is not required for mice to pursue moving prey, this result does not exclude a role of DS circuitry in predictive direction coding. Live prey, such as crickets, move dynamically and can unexpectedly undergo periods of non-movement or periods of stochastic, non-predictive movement. In cases such as these, the stimulus of interest (i.e., prey) either has no motion, or its motion direction is random. In these cases, the ability to compute motion direction could either have no impact on or add noise to a system designed to detect or track stimuli of interest. In response to smooth motion, however, the direction in which a stimulus of interest moves could be valuable to the animal and assist in generating predictive directed eye movements (Horwitz and Newsome 2001; Ma et al. 2013). Mice will approach artificial visual stimuli that approximate the spatiotemporal properties of live prey (Procacci et al. 2020). Therefore, simple, prey-like visual stimuli that vary in how predictive their movement is could be generated for mice to interact with. Similar approaches to those utilized in Chapter 2 of this dissertation could then be implemented to test whether there are systematic differences in the approach frequency of mice 1) lacking retinal DS and/or 2) whose various DS-encoding visual pathways have been silenced.

Convergence in NF cells

NF cells sample diverse RGC inputs that encode a small area of retinotopic space (Gale and Murphy 2014). By sampling visual inputs that are distributed vertically in sSC, NF cells therefore might be innervated by RGCs encoding different features of visual space that are represented at different depths of sSC (i.e., DS, object motion, contrast-encoding, and
illuminance-encoding) (Gale and Murphy 2014; Cang et al. 2018; Dhande and Huberman 2014). Based on the superimposed retinotopic mapping of SC (Cang et al. 2018), ipsilateral inputs encoding the binocular visual field might also be integrated with a variety of contralaterally projecting RGCs that encode visual features. Our current results cannot prove the integration of these varied inputs onto single NF cells in sSC; however, within a single retrograde tracing study (i.e., tracing inputs to many starter NF cells), presynaptic RGCs were identified in both the ipsilateral and contralateral retinas, suggesting an overarching mixing of RGC inputs onto this population of cells.

At the single-cell level, a similar approach could be implemented as was reported by (Rompani et al. 2017) to retrogradely label and physiologically characterize the combination of contralaterally and ipsilaterally projecting RGCs that innervate individual NF cells. While 4 cell types might be sufficient to morphologically and physiologically characterize the cell types in sSC, cells belonging to a single class (i.e., NF cells) are not functionally identical (Gale and Murphy 2014) and are unlikely to have homogeneous inputs. An understanding of the variability with which ipsilaterally and contralaterally projecting RGCs with different response properties target single NF cells would be valuable to understanding how their properties are integrated to tune NF cells to small, fast-moving objects moving in a particular direction (Gale and Murphy 2014; Hoy, Bishop, and Niell 2019).

At a functional level, it remains unclear how the response properties of NF cells suit them to participate in the pursuit and capture of prey. Their DS tuning is not required for reliable pursuit. Does this indicate that another feature is more important to execute this behavior (i.e., size or speed tuning; putative visual acuity conferred by ipsi-αRGCs)? Or could this indicate that NF cells integrate inputs from multiple RGCs to reach a spike generation threshold to execute motor
commands? In vivo electrophysiological characterization of NF cells whose presynaptic inputs have been manipulated (i.e., ooDSGCs rendered non-DS or ipsi-αRGCs silenced) would be valuable to ascertain how stimulus information encoded by these inputs is used to generate the behaviorally relevant outputs encoded by NF cells. Absent DS inputs, what functional characteristics of NF cells remain intact that might preserve a mouse’s ability to pursue and capture prey? In contrast, without ipsi-αRGC input, how do NF cell responses change that can disrupt predation?

4.3 References


