## VISUAL CONTROL OF HOVERING IN ANNA'S HUMMINGBIRDS

by

## **BENJAMIN GOLLER**

## B.Sc., University of Utah, 2008 M.Sc., University of California in Riverside, 2011

## A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

## DOCTOR OF PHILOSOPHY

in

# THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES (Zoology)

## THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

December 2016

© Benjamin Goller, 2016

## ABSTRACT

Relatively little is known about how sensory information is used for controlling flight in birds. A powerful method is to immerse an animal in a dynamic virtual reality environment to examine behavioral responses. The research comprising this dissertation investigated the role of vision during free flight hovering in hummingbirds to determine how optic flow -image movement across the retina- is used to control body position. We filmed hummingbirds hovering in front of a projection screen with the prediction that stationary patterns would allow a hummingbird to maintain stable body position, but moving patterns would change hovering stability. When hovering in the presence of moving gratings and spirals, hummingbirds lost positional stability and responded in the direction of the stimulus motion. There was no loss of stability with stationary patterns (Chapter 1). How sensitive are hummingbirds to visual motion? We predicted that small changes in the direction of a looming motion would result in matched changes in backward flight response of hummingbirds. Providing stationary visual patterns in combination with looming spirals was predicted to rescue hovering stability. Our results suggest that hummingbirds are not only sensitive to small changes in motion direction, but also sensitive to any visual motion of the background, even when large stationary features are present (Chapter 2). The sensitivity of hovering hummingbirds to visual motion suggested that other senses might be involved to stabilize flight. When docked with a feeder, hummingbirds gain a stable physical reference through bill contact. We predicted that tactile feedback during docked feeding would provide the necessary stationary reference to help hummingbirds override their sensitivity to visual motion. We built an instrumented feeder that measured how much a docked hummingbird pushed laterally and vertically. Hummingbirds were not very precise during docked hovering and pushed against the feeder in an attempt to stabilize left, right, and downward visual motions.

ii

Upward motion was not matched by pushing against the feeder (Chapter 3). Collectively, these experiments demonstrate that hummingbirds control hovering position by stabilizing motions in their visual field both when hovering in space and when docked with their bill inserted into a flower.

## PREFACE

All of the work presented henceforth was conducted in the Altshuler laboratory in the Department of Zoology at the University of British Columbia, Vancouver campus. All experiments were performed with approval by the University of British Columbia Animal Care Committee [protocol #A15-0116].

A version of chapters 1 and 2 has been published in a joint manuscript [Goller, Benjamin and D. L. Altshuler. 2014. Hummingbirds control hovering flight by stabilizing visual motion. Proceedings of the National Academy of Sciences USA 111(51): 18375-18380]. I was the lead investigator, responsible for all components of the project including concept formation, experimental rig design, data collection and analysis, as well as manuscript composition. D. L. Altshuler was the supervisory author on this project and was involved throughout the planning, experimental, analysis, and writing phases of the project.

The work in chapter 3 is original and currently unpublished. A version of chapter 3 has been prepared for submission to Frontiers in Neuroscience in the Perception Science section as part of a special research topic titled "Visual Control of Avian Flight". I was the lead investigator, responsible for all major areas of concept formation, experimental rig design, data collection, and statistical analysis, as well as manuscript composition. D. L. Altshuler was the supervisory author on the project and involved throughout the planning, experimental, analysis, and writing phases.

## TABLE OF CONTENTS

Abstract	ii
Preface	iv
Table of Contents	v
List of Tables	vii
List of Figures	viii
Acknowledgements	X
Introduction	
Chapter 1 - Hummingbirds control hovering flight by stabilizing	visual motion11
Introduction	
Methods	
Results	
Discussion	
Figures	
Chapter 2 – Hovering hummingbirds respond to small changes in	background looming
motion	
Introduction	
Methods	
Results	
Discussion	

Figures	
Chapter 3 – Hovering hummingbirds attempt to stabilize visual	motion during docked
feeding	
Introduction	
Methods	
Results	
Discussion	
Figures	
Conclusion	
References	

## LIST OF TABLES

Table 1. Parameter estimates for series of combined patterns with either	
increasing looming spiral or increasing stationary pattern	62

## LIST OF FIGURES

Figure 1. Anna's hummingbirds respond to moving visual patterns during
free flight hovering
Figure 2. Rotating spirals and moving gratings caused hummingbirds to
lose positional stability in the orientation of the motion but stationary
patterns did not affect hovering
Figure 3. Representative traces of hummingbird movement demonstrate how
docked feeding and undocked look-ups differ depending upon spiral motion27
Figure 4. Representative traces of hummingbird movement demonstrate how
docked feeding and undocked look-ups differ depending on motion of a
vertical grating
Figure 5. Representative traces of hummingbird movement demonstrate how
docked feeding and undocked look-ups differ depending upon motion of a
horizontal grating
Figure 6. Pairwise comparisons for movement along the x-axis demonstrate
that looming and receding motion elicit strong backward and forward flight
responses
Figure 7. Pairwise comparisons for movement along the y-axis demonstrate
that right and left visual motion elicit strong right and left flight responses35
Figure 8. Pairwise comparisons for movement along the z-axis demonstrate
that upward and downward visual motion elicit strong upward and
downward flight responses
Figure 9. Frequency of look-ups and feeding duration are similar among
different stimulus treatments

Figure 10. The position of hummingbirds hovering near a projection screen
was measured in 3-dimensions
Figure 11. Hummingbirds shift the orientation of their backward flight to
match offsets in the position of the looming spiral
Figure 12. Moving the looming spiral center left, right, up, or down causes
the radius of the flight response to decrease relative to a centered spiral60
Figure 13. Even with a prominent stationary pattern, a looming spiral
produces the characteristic avoidance response, which increases with an
increased proportion of looming spiral in the projected image
Figure 14. Experimental rig was designed to measure how hovering
hummingbirds interact with the feeder
Figure 15. Docked hummingbirds interact with the feeder
Figure 16. Hummingbirds attempt to stabilize visual motion during docked
feeding, especially in the horizontal axis
Figure 17. Hummingbird attempts to stabilize leftward visual motion exhibit
speed tuning

### ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Doug Altshuler, for his mentorship and support throughout my graduate work. He gave me the freedom and the resources to pursue questions and topics that I found exciting and interesting, but challenged my ideas and kept me moving forward when direction was needed. Guidance was also provided by my committee: Jim Enns, Darren Irwin, and Bob Shadwick, who have patiently provided feedback and advice on countless drafts and presentations, and my work is greatly improved because of their contributions. The work in this dissertation was also possible because of the collective efforts of many past and present members of the Biomechanics group at UBC, who assisted my research efforts on a daily basis and provided feedback and support throughout the course of my experiments.

There are also countless others to thank for their ideas, questions, suggestions, and help throughout the course of my time at the UBC. Caring for hummingbirds, designing experimental rigs and experiments, and troubleshooting methods for recording and analyzing behavior were all accomplished with the help of many people, too numerous to list here. Science is a collaborative effort and I had the good fortune to interact with many people who took the time to share their insights and skills with me, and my research is better because of their contributions.

Finally, I would be remiss, if I did not acknowledge the support of friends and family, who continue to endure my stories about hummingbirds and spirals. Along with Langley United Sparta, BoZo, and the exploding local beer scene, it was my family and friends that provided the much needed relief from hours at the computer. The few shreds of sanity I can still claim at the end of my graduate career are courtesy of the culinary exploits, outdoor adventure, and fermented creations I enjoyed with those individuals. Thank you, and cheers!

Х

## INTRODUCTION

Moving through a complex, natural environment requires rapid transformation of sensory information into instructions to the muscles that will then produce appropriate body motions (Dickinson et al., 2000). Flight is an especially demanding form of locomotion from both a motor and a control perspective. In the absence of the passive stability afforded to terrestrial and aquatic animals by ground reaction forces and buoyancy respectively, the flying animal must constantly monitor its position in the environment, both to guide the animal's behavior and to prevent catastrophic loss of stability. To handle this challenge, flying animals use multiple senses to carefully monitor changes in their position and orientation (Taylor and Krapp, 2007). Vision is especially important to flying insects and birds. As the animal flies it perceives motion because visual projections of the environment onto the retina move, a phenomenon termed optic flow.

Optic flow has been shown to be very important for control of behavior in flying insects, but relatively little was known about how birds use visual motion to control flight. To put this dissertation into context, this introduction will review motion detection and how visual motion is important for insect behavior and neuroscience. Next, advances in vertebrate neuroscience are discussed, with an emphasis on our understanding of visual motion processing in birds. The visual motion processing pathways in the avian brain have been well-studied, and have generated hypotheses about visual control of flight in birds, including control of hummingbird hovering. Hovering therefore falls at an important intersection between avian neuroscience and insect-like flight that makes hummingbirds a candidate for new investigations of visual motion, and its importance for the control of avian flight. This dissertation focuses on hovering in Anna's hummingbirds (*Calypte anna*) and presents a series of experiments investigating how visual motion is used for body position control during free-flight hovering and hover feeding.

Perception of visual motion is important for many behaviors (Gibson, 1954, 1958; Lee, 1980; Lee and Lishman, 1977). Individual photoreceptors within an eye only detect changes in the input light flux, but an array of receptors with associated neural circuits is capable of encoding a complex pattern of translations and rotations (Nakayama and Loomis, 1974). Motion elements comprising a visual motion, or optic flow, pattern are perceived by comparing information from two spatially distinct receptors, which allow detection of motion, and also of the direction of that motion (Reichardt, 1986, 1987). This simple detector, dubbed the elementary motion detector (EMD), can be repeated many times in a compound eye, or retina, but is limited in that it cannot distinguish the relative contributions of spatial (cycles per degree) and temporal (degrees per second) frequencies to the perceived velocity (cycles per second). Although alternative motion detector models that are better suited to measure velocity have been proposed (Zanker et al., 1999), the EMD's ambiguous velocity coding can also be viewed as a feature of motion perception that incorporates textural information (Egelhaaf et al., 2014). Many subsequent studies have investigated eyes, motion processing neurons, and visually guided behaviors with this detector, and its properties, in mind.

The importance of vision, specifically the detection of optic flow, for controlling numerous components of flight behavior has been demonstrated in many insect studies. As expanding motion (looming) reaches a certain threshold level in flies (and other insects), they extend their legs in a landing response (Braitenberg and Ferretti, 1966; Coggshall, 1972; De Talens and Ferretti, 1970; Tammero and Dickinson, 2002a). However, flies also steer or perform a rapid turn – a saccade – away from looming features that originate in lateral portions of the visual field, suggesting that the origin of the looming motion in the visual field informs the decision between landing and collision avoidance (Tammero and Dickinson, 2002a). In general,

turning saccades are also elicited by visual motion, which then, together with halteres, provides some of the feedback for terminating the saccade (Bender and Dickinson, 2006a, 2006b; Tammero and Dickinson, 2002b). When flying to a food source, bees measure distance using visual motion so that they can communicate distance to a food source to their nest mates. While the motion perception is described by EMD models, the sophisticated integration used to make distance calculations is not understood (Srinivasan et al., 1996, 1997). Behavior is also limited by the EMD, which cannot directly measure velocity of a moving visual pattern, so behavioral responses such as turning in flies instead are tuned to the temporal frequency of visual motion (Srinivasan et al., 1999).

Hovering is a locomotor behavior that presents an interesting visual scenario for the flying animal – the absence of an optic flow field. In addition, unlike some of the other flight behaviors, where a response is difficult to predict and measure, it is simple to define changes in hovering because the animal is stable in space under normal conditions. Introducing a visual motion is predicted to produce a matched stabilization response characterized by the hovering animal changing its position in the same direction as the visual motion. Visual motion could be the result of objects moving relative to the hovering observer, for example an approaching predator, which would most likely warrant a different behavioral response, such as abrupt escape flight. A large and coherent optic flow field however, is likely the result of the observer moving so body position should be adjusted slowly to minimize the background motion perceived on the retina. Guard bees that normally hold stable positions at the entrances of their nests fly away from centers of visual expansion, or follow translational visual motion, until they lose visual contact with the entrance they were guarding (Kelber and Zeil, 1990, 1997). Hawk moths regulate their distance from a flower visually, and improve their ability to maintain distance

when the 'flower' has a pattern that provides visual contrast (Farina et al., 1994) or when the environment is brighter (Sponberg et al., 2015). The moths also track background motion, but only during contact with the flower, otherwise they may even fly against the visual motion (Kern and Varjú, 1998). These experiments, as well as numerous others with insects that attempt to hold a position in space (Collett, 1980; Junger and Dahmen, 1991) suggest that visual motion is an important error signal when maintaining a stationary position in an environment that is assumed to be stationary.

Insect systems have also provided models for testing perception of motion that would be used to control a behavior like hovering. For example, the visual processing region of the head ganglion in flies is organized into four layers where the neurons themselves are organized in retinotopic fashion, matching the structure of the retina (Borst et al., 2010). Lobula plate tangential cells (LPTCs) have extensive dentritic projections that suggest they receive numerous inputs from the visual system, as was predicted for an array of EMDs, and have specific sensitivities to different parts of the visual field or different motions (Borst and Haag, 2002; Krapp and Hengstenberg, 1996). In addition, connections between LPTCs allow for motion specificity in single cells to be combined to generally encode motion in a receptive field (Borst et al., 2010). To affect the motor system, information must be sent to the thoracic ganglion, and descending neurons of the ocellar and vertical system serve to encode self-motion cues along preferred axes (Wertz et al., 2009).

Recently, new imaging and genetic technologies in model systems like *Drosophila* are enabling increasing resolution of the activity of neurons to motion stimuli including neuron responses to lightening and darkening changes that underlie basic, contrast dependent, motion detection (Hopp et al., 2014; Strother et al., 2014). Studies of motion processing and the

underlying neurons are also providing insight into how spatial information is coded, providing new perspectives on navigation through cluttered environments (Egelhaaf et al., 2014). Key parts of the motion detection model and flight control networks remain to be described on a neuron level. These important processes or cells may be buried in other layers of the head ganglion or in complex interactions between neurons (Borst et al., 2010; Srinivasan et al., 1999).

In addition to gaps in our understanding of how EMDs are implemented in the brain, some behaviors have been shown to be visually mediated but appear to require velocity coding that is not provided by EMDs. Velocity control in bees and bumblebees depends on maintaining a set image velocity on the retina, and incorporates both lateral and ventral visual fields as well as flexibly incorporating different portions of the frontal visual field (Baird et al., 2006, 2011; Linander et al., 2016; Srinivasan et al., 1996). Flight altitude is similarly tied to ventral image velocity, with flies and bees flying lower to the surface when optic flow cues from the ground are weak (Baird et al., 2006; David, 1979). By comparing the lateral velocity of motions in the left and right eye, bees are able to maintain a centered flight path between two surfaces, but measuring those visual motion velocities appears to be immune from classical EMD limitations (Srinivasan et al., 1999). To understand more complex behaviors, studies of free flight seek to combine sophisticated tracking with modeling or visual reconstruction to allow flight trajectories and visual input to be related (van Breugel and Dickinson, 2012; Mronz and Lehmann, 2008). Furthermore, laboratory studies must be validated in natural or semi-natural scenarios because flight behaviors and neuron responses are more robust in experiments using natural scenes which have complex spatial frequency structures (Baird and Dacke, 2012; Barnett et al., 2010; Straw et al., 2008).

Behavior control is a complex integration of motor and sensory information, so exploration of how visual information is used to perform a specific flight control task is only one approach to understanding flight. Animals tend to combine a series of tasks to produce a single flight trajectory or behavior and we are currently describing the numerous algorithms handling visual information processing for different components of such a flight (Srinivasan et al., 1999). In addition, many external cues modify behavior through other sensory systems (Chow and Frye, 2008; Frye, 2010; Frye et al., 2003; Wasserman et al., 2015; Willis et al., 2011). How does the brain structure these components to produce complex flights using a single set of wings and a single array of sensors? Understanding the transitions between flight components and the sensory processing and prioritization that allows an animal to perform behavioral tasks when faced with complex sensory signals is an important next step.

Vertebrate systems are starting to gain favor for these types of behavior control studies as advances in sensors and neural probes enable more instrumentation of free behavior. Although vertebrate fliers are not model systems with genetic technology comparable to *Drosophila*, recent high-profile studies of behaving bats have led to breakthroughs in our understanding of spatial mapping in the hippocampus (Finkelstein et al., 2015; Ulanovsky and Moss, 2007; Yartsev and Ulanovsky, 2013) providing insight into vertebrate control that is unlike anything seen in insects. Avian and mammalian visual motion processing circuits are well described and compared (Wylie et al., 2009), and more recent work is focusing on cerebellar integration in avian models (Wylie, 2013). Interestingly, studies of avian behavior have lagged behind the neural advances. The stage is therefore set for the integration of neuroscience research with behavior, comparable to the body of work that describes behavior control in several flying insects.

Birds are highly visual animals (Zeigler and Bischof, 1993) with sophisticated vertebrate eyes, and there are numerous examples of avian taxa with enhanced visual acuity, color vision that extends into the ultraviolet range (Chen and Goldsmith, 1986; Ödeen and Håstad, 2010), and retinas with multiple specializations and/or foveae (Lisney et al., 2015; Wood, 1917). Considered among the most visually dependent animals, birds also have large brain regions devoted to three visual information processing pathways, called the accessory optic system (AOS), and the tectofugal and thalamofugal pathways (Wylie et al., 2015). The tectofugal pathway involves visual processing in the optic tectum and deals primarily with object motions relative to the observer (Wylie et al., 2009). The thalamofugal pathway is important for detail discrimination and fine analysis of the visual field and involves an important processing center named the Wulst (Zeigler and Bischof, 1993). Finally, the AOS is a more diffuse pathway that has several regions with neurons that respond to large motion patterns such as those generated when the observer is in motion (Frost et al., 1990). These pathways have regions and functions defined by identifiable neurons that specialize for parts of the visual field or for specific motion types (Frost et al., 1990; Wylie et al., 2009). For example the pigeon nucleus rotundus in the tectofugal pathway has classes of neurons that respond to different parameters of object looming motion, however they do not respond if the pigeon is moved toward the object to create similar visual expansion (Sun and Frost, 1998).

Large visual flow fields, such as those generated by observer motion, are processed primarily in the accessory optic system. Much like the visual neurons of insects, motion direction and speed preferences are characteristic of AOS neurons in two important nuclei: the lentiformes mesencephali (LM) and the nucleus of the basal optic root (nBOR), which are interconnected and complementary nuclei (Morgan and Frost, 1981; Wylie and Frost, 1990, 1999). The

distribution of preferred directions and speeds, as well as overall volume of these two pre-tectal motion processing nuclei provide clues about visual motion and its importance for avian flight control. The LM of birds, as well as the homologous nucleus of the optic tract (NOT) in mammals, exhibits a temporonasal (back-to-front) preference, and this matches optimal directions for head and eye stabilization reflexes (Eckmeier and Bischof, 2008; Gioanni, 1988a). The LM is also enlarged in hummingbirds, and to a lesser extent in transiently hovering avian species such as the kingfisher, suggesting a link between hovering and the temporonasal preferring LM (Iwaniuk and Wylie, 2007). Interestingly, the complementary nucleus, nBOR, which prefers nasotemporal, upward, and downward translational motion (Morgan and Frost, 1981; Wylie and Frost, 1990) is not enlarged.

Hummingbirds (family *Trochilidae*) are a clade of nectivorous birds that are specialized for hovering and have brains that may be specialized for visual motion processing to facilitate hovering. These small hovering birds are specialized in numerous other ways and exhibit physiological (Suarez et al., 1990; Welch et al., 2006), morphological (Greenewalt, 1962; Mathieu-Costello et al., 1992; Suarez et al., 1991), and neuromuscular (Donovan et al., 2013; Gaunt and Gans, 1993; Hagiwara et al., 1968; Tobalske et al., 2010) specializations required to generate and control the high and sustained power output (Chai and Dudley, 1995; Wells, 1993) required for their energetically expensive hovering flight mode (Clark and Dudley, 2010). In addition, the bill of hummingbirds has become elongated to allow the birds to access the small quantity of nectar in a flower (Wolff, 2006) with some birds even exhibiting bill shapes that match specific corollas (Stein, 1992). The tongue is also specialized to trap nectar so that the bird can feed by extending and retracting its tongue (Rico-Guevara et al., 2015; Rico-Guevara and Rubega, 2011). It is clear that hummingbirds are specialized to interact with flowers, much like insects, but the extent to which they control their insect-like hovering flight using visual motion, instead of vestibular feedback and other sensory information, had not been explored.

Quantitative description of a hummingbird's approach to a flower suggests that optic flow, specifically visual expansion, could be used to control deceleration and docking (Lee et al., 1991) much like a landing insect or bird (Baird et al., 2013; Bhagavatula et al., 2009; Lee et al., 1993). This type of behavior does not specifically match the motion preference of the proposed specialized LM nucleus, but hovering may provide an insight into the role of visual motion processing in hummingbird flight control. By studying hummingbirds, naturalistic flight behavior of a flying vertebrate can be produced in the laboratory and the sensorimotor control of avian flight behavior can be studied in a controlled sensory environment. Neurophysiology suggests parallels between the properties of insect and avian visual motion processing neurons, but birds have been the subjects of relatively few behavioral studies.

Hummingbirds are the only avian taxon specialized for hovering, which places them at an intersection between an otherwise "insect-like" flight mode and a vertebrate sensor suite and processing center. In concert with the morphological and physiological characteristics of hummingbirds, neuroanatomy suggests that their brains have enlarged regions that deal with visual information relevant for their nectar feeding ecology (Iwaniuk and Wylie, 2007; Ward et al., 2012). However, the functional flight control connection between the information collected by the hummingbird sensor suite and hover-feeding behavior has not been investigated. The Anna's hummingbird (*Calypte anna*) provides an excellent opportunity to investigate sensorimotor integration in a flying vertebrate.

This dissertation research investigated questions about the influence of sensory information on station-holding behavior by measuring behavioral changes associated with a

moving visual environment. First we studied whether hummingbird ability to maintain a stationary position in space during hovering is affected by visual motion. We measured the position of hovering hummingbirds in a flight arena where we could present the free flying birds with moving background patterns (Chapter 1). Hummingbird responses to visual motion were then probed further to determine hummingbird sensitivity to small changes in motion direction and whether stationary patterns would help hummingbirds maintain position (Chapter 2). Finally, docking with a feeder introduces a physical reference to hovering flight because the bill is in contact with a stationary structure. We also tested the prediction that hummingbirds are stable and precise during hovering at flowers by measuring how much a hummingbird bumps and pushes against an instrumented feeder. We characterized normal hummingbird-flower interactions, then presented feeding birds with visual motion patterns to determine if docked hummingbirds attempt to move in the direction of visual motion (Chapter 3). These experiments represent an in-depth study of the effect of moving visual patterns on un-docked and docked hovering position stability in hummingbirds and provide behavioral support for hypotheses derived from neuroanatomical studies that predict hummingbird sensitivity to visual motion during hovering.

## CHAPTER 1 - HUMMINGBIRDS CONTROL HOVERING FLIGHT BY STABILIZING VISUAL MOTION

### Introduction

To precisely control their motion through the air, flying animals have evolved specialized sensory structures and associated neural architecture. Neural specializations provide hypotheses for what senses are most important to a given taxon and although flight control has been studied extensively in insects (Taylor and Krapp, 2007), birds have until recently received limited attention. Birds have large regions of the brain dedicated to visual processing, suggesting parallels with insects such as a leading role for optic flow in controlling flight paths (Frost, 2010; Mronz and Lehmann, 2008). It has recently been demonstrated that birds exhibit visually mediated position control much like bees (Bhagavatula et al., 2011; Srinivasan et al., 1991) even though they have complex spatial mapping in the hippocampal formation, and a much larger brain for interpreting visual input (Ward et al., 2012) and dynamically integrating vision with proprioceptive and vestibular feedback (Fetsch et al., 2009; Lappe et al., 1999; Pelah and Barlow, 1996). Further work with birds is required to determine the extent of insect and avian visual flight control convergence.

In birds and mammals, the visual information from the eyes is divided into three separate pathways that each process a subset of motions or visual features (Frost, 2010; Wylie et al., 2009). Two of these pathways, named the accessory optic system and tectofugal pathways in birds, each process a single type of motion: 1) self or ego-motion is the motion produced when an observer moves relative to their environment and 2) object motion when visual features move

relative to the observer (Frost, 2010). Using the same retinal information, the visual system of a flying hummingbird must separate motions arising from the bird moving through foliage toward a flower from the motion caused by an approaching competitor or predator. During hovering, the hummingbird similarly must determine if visual motion is caused by positional instability, where the observer moves relative to a stationary background feature, or by background motion that is independent of the hovering observer. Is visual motion processing, and the potential complexity of separating moving objects and self-motion, important for control of body position in hovering hummingbirds?

In natural settings, hummingbirds are able to precisely hover in place, even though natural settings are rarely devoid of visual motion in the background. Hummingbirds could hold a stable position using a variety of sensory information, including monitoring optic flow. Here, we test the hypothesis that vision is a major source of sensory feedback for stabilizing body position during hovering in hummingbirds, similar to hovering control in insects (Collett and Land, 1975; Farina et al., 1994; Kelber and Zeil, 1990). Specifically, we examine the role of vision in avian flight control by testing three predictions in a free-flight laboratory arena: hovering hummingbirds will 1) be stable during hovering when the background is stationary, 2) will drift during hovering when the background is moving, and 3) drift direction will match the direction of background motion in all directions.

#### Methods

Animal model: Experimental subjects were eight male Anna's hummingbirds, *Calypte anna*, that were trapped on the University of British Columbia campus (caught November 5, 2012-October 29, 2013). Hummingbirds were individually housed in 0.61 x 0.61 x 0.91 m cages and fed *ad libitum* sugar (15% by weight) or Nektar-Plus (Nekton, 13% by weight) solution. Individuals were given three days to get used to captivity and the laboratory setting before training began in the experimental chamber with a feeding schedule. The feeder, filled with sugar solution, was closed to prevent feeding between experimental trials, and opened at intervals of either 15 or 20 minutes depending on the experiment. When the feeder was opened, birds were allowed to feed until they departed. Restricting food between feeding was important to increase the time birds spent at the feeder. All experiments were performed with approval of the University of British Columbia Animal Care Committee.

**Experimental rig:** The experimental chamber was a large clear acrylic cylinder (Fig. 1A; 0.70 m diameter, 0.61 m height). A 0.91 m, full-height section of the wall (41.4% of circumference) was covered on the outside by a frosted window coating (wallpaperforwindows.com) allowing back projection onto the cylinder. We used a Canon REALiS SX80 Mark II projector (3000 lumen lamp, 1400x1050 SXGA, 60Hz) to display the stimuli. This projector uses Liquid Crystal on Silicon (LCoS) technology so there is no flicker or dark phase in the projection as one would have with a projector that has a spinning color wheel. Visual stimuli were generated and controlled using custom scripts in VisionEgg (Straw, 2008).

At the center of the projection screen was a small hole where a clear plastic feeder was attached such that it extended 0.175 m into the chamber. The ceiling of the chamber was nylon mesh with holes for the lenses (Computar H2Z0414C-MP) of three cameras (Prosilica GigE680,

Allied Vision Technologies). These three cameras were used to auto-track a painted white spot on the top of the bird's head using Flydra 3D tracking software (Straw et al., 2011). Filming was conducted at 100 frames per second (fps) and the cameras were synchronized by an external trigger. The tracked flight trajectories were converted into 3D (x,y,z) coordinates (Fig. 1B) and exported for further analysis using custom scripts in Matlab (Mathworks R2012a).

**Stimulus description:** The spiral pattern was produced using Matlab to draw a four-armed 10 degree logarithmic spiral. Areas between the four spiraling lines were filled with black and white in alternating fashion. The same spiral pattern was rotated in either clockwise or counterclockwise direction at a rotation frequency of 0.5Hz. For our spiral pattern and rotation frequency, clockwise rotation (looming) corresponds to a time-to-collision ( $\tau$ ) value of 1.86 seconds. For a counterclockwise (receding) spiral the analogue of  $\tau$  is the time to double distance but has the same time value (Martinoya and Delius, 1990). We tested the luminance of the projected spiral image. White spiral segments ranged from 316 to 160 cd/m<sup>2</sup> from the center of the spiral to the periphery. Black segments measured 87 cd/m<sup>2</sup> in the center and 35 cd/m<sup>2</sup> at the periphery.

The linear gratings used in these experiments were black and white bars moving at temporal frequency 0.5Hz (cycles per second) either left and right for vertical bars, or up and down for horizontal bars. The spatial frequency of these linear gratings was 0.044 cycles per degree. We selected this spatial frequency because it produced gratings with the same number of cycles as encountered when moving radially outward from the center of the spiral pattern.

**Experimental protocol:** The experiment relied on filming during a prolonged feeding bout which could be performed roughly every 20 minutes with a trained hummingbird. Long feeding durations were key to maximize the number of pauses (look-ups) during feeding where the

hummingbird hovered near the feeder but was un-docked. Access to food was restricted by removing the feeder between trials and any moving stimulus patterns were stopped. Trials stopped when a bird finished feeding (left the filming area around the feeder) or after two minutes without a bird approaching the feeder. Restriction of feeding bouts, combined with a 12% by weight sucrose solution, increased the feeding duration relative to feeding flights when hummingbirds were given *ad libitum* access to higher sugar concentrations.

We tested the free flight response to forward-backward, lateral, and vertical visual motion, using three background black and white patterns: a spiral, vertical grating, and horizontal grating. The spiral was either rotated clockwise or counterclockwise to produce constant looming or receding motion respectively. The vertical gratings were moved either left or right and the horizontal version was moved up or down. In addition, each pattern had a stationary (no-motion) treatment for a total of nine treatments. Hummingbird subjects were exposed to a randomized series of these treatments with every stimulus shown twice (18 trials per individual, 8 individuals total) over the course of a single day. Any feeding flight where the hummingbird consumed <0.25 milliliters of sugar solution was discarded and the trial was repeated until the minimum consumption level was surpassed, which was generally after a single repeat.

**Measuring response to visual motion:** Flight response measurements were generated by custom Matlab (Mathworks R2012a) analysis scripts. Representative traces for feeding bouts in the presence of moving and stationary visual patterns (spirals and gratings) are presented in figures 3-5. A bird's position and velocity were used to define two types of hovering behavior, which could each appear multiple times within a single feeding bout. *Docked feeding* segments are defined by the bird being within physical reach of the front of the feeder and having a net velocity less than 0.050 meters per second. *Undocked look-up* segments are defined by the bird

not being within physical reach of the front of the feeder but still having a net velocity less than 0.050 meters per second. Only segments that lasted at least 0.25 seconds were included in analyses.

For each segment of docked feeding or undocked look-ups we calculated the average movement in six directions along three axes. The three axes (x,y,z) were defined globally with respect to the feeder with x as forward-backward, y as left-right, and z as down-up. Movements in the forward, left, and down directions were defined as negative, and movements in the backward, right, and up directions were defined as positive.

We first calculated the derivative of the bird's position along each axis. These derivatives were then sorted by motion direction (positive vs. negative values within a single axis) and all values pertaining to a single motion direction were summed to get a total distance traveled in each of the six directions. We next normalized the summed movement by dividing it by the duration of the segment. Thus, movement in each direction is represented as movement (in centimeters) normalized to one second of flight.

Movement in each of the six directions was analyzed separately in R (Pinheiro et al., 2016; R Core Team, 2015). Post-hoc testing to compare different direction treatments was conducted with the multcomp package (Hothorn et al., 2008). Six identical linear mixed effects models with stimulus as a fixed effect and individual bird as a random effect were used to test for the effect of each stimulus level (9 levels total) on response motion in each of the six directions measured. Tukey tests were then performed to examine pairwise comparisons of stimulus types within individual axis directions. We analyzed several additional measures to generally describe the behavior of the hummingbirds during stimulus trials. Feeding duration (time in docked

position) and the frequency of look-ups (number of look-ups divided by total duration) in each trial were analyzed in the same way.

#### Results

The free flight responses of Anna's hummingbirds (*Calypte anna*) to different optic flow cues were studied in a circular chamber that included a projection screen. Feeding bouts were composed of two intermittent phases, docked feeding and undocked look-ups during which the bird withdrew from the feeder and hovered in front of it. When there was a non-moving pattern on the screen, the head position was stable during both docked and undocked phases (Fig. 1B-C, Figs. 3-5 B). A looming stimulus elicited oscillations in head position during docked feeding, and elicited non-oscillatory backward drifts during look-up phases (Fig. 1D, Fig. 3A). Both responses are consistent with the prediction that looming motion produces a matched backward avoidance response. Rotating the same spiral in the opposite direction causes receding visual motion. In this case, the feeder physically blocked forward motion during docked feeding, so we decided to focus only on body position during the undocked look-ups for further analysis (Fig. 3C). We provide all of the raw traces of drifts during look-ups in the x-axis for one representative bird in response to looming, receding, and stationary spirals (Fig. 1E).

In addition to looming and receding visual motion, we also present the flight responses of hummingbirds to left, right, down and up motion, caused by vertical and horizontal linear gratings. Raw traces of responses to all motion types for one individual are provided in figures 3-5. The complete set of look-up phases that were extracted from all eight individuals and all stimulus treatments is provided in figure 2 (A-D). In all treatments without pattern motion, the

magnitude of drifts during look-ups was relatively small, and exhibited a forward tendency. The average look-up response to treatments with pattern motion (Fig. 2E-F) illustrates that hovering hummingbirds 1) are stationary when the background is stationary, 2) respond to large, moving visual patterns by drifting, and 3) drift along all three global axes primarily matching the direction of stimulus motion.

Stimuli had a significant effect in all cases (all  $F_{8,106} = 23.619$  to 86.009, all *P* < 0.0001) and the results of all tests indicate that during undocked look-ups the hummingbirds exhibit an increase in movement in the direction of the stimulus pattern motion (Figs. 6-8). Given our prediction that drift direction would match stimulus direction, the corresponding comparisons between the direction of the stimulus and all other directions have been highlighted in figures 6-8. During docked feeding, strong oscillatory movement is seen with a looming spiral stimulus (Fig. 6) but we did not analyze these movements further for this experiment because we were unable to measure docked feeding responses with other motion directions. The rigid feeder limits the ability of a bird to move laterally and vertically, as well as forward, once docked. However, the presentation of a moving stimulus could also affect the overall feeding behavior so we analyzed how often birds stop feeding to look up, and how long the feeding flights lasted.

There is considerable variation among individuals in the frequency of look-ups and in their feeding duration (Fig. 9). We did measure an effect of stimulus treatment on look-up frequency ( $F_{8,128} = 2.766$ , P = 0.0075), but this was the result of a single significant Tukey contrast, between looming and receding spiral treatments (estimate: 0.161, P < 0.01). No other contrasts yield a significant difference in look-up frequency (estimates: -0.109 to 0.105, all P >=0.0872, Fig. 9A). Stimulus treatment did have a significant effect on feeding duration ( $F_{8,128} =$ 4.658, P < 0.0001). Birds fed for shorter durations in the presence of a receding spiral (estimates:

-9.051 and 6.912 to 9.074, all P < 0.01), except compared to a no-motion spiral (estimate: -5.388, P = 0.080). All other stimulus comparisons were not significant (estimates: -3.663 to 3.687, all P > 0.537, Fig. 9C).

### Discussion

Collectively these results indicate that hovering hummingbirds are highly sensitive to the direction (Figure 2) of visual pattern motion when presented with large coherent stimuli, and hold a stationary position in the absence of visual motion. Behavioral responses to optic flow, called optomotor responses, are common across animal taxa, and we demonstrate that hovering hummingbirds will respond to visual motion in three global axes by changing their body position. Our results show that freely hovering hummingbirds drift in a moving visual environment, primarily to match the direction of background motion, and do not drift when the motion is absent.

Our study has limited resolution for comparing the responses to different motion axes or directions. We measured only the position of the head in space and do not have the ability to dissociate eye, head, or body movement components of the overall response to our stimuli. There is reason to predict that hummingbirds would have optomotor gains that are stronger in some directions than others based on experiments with birds (Eckmeier and Bischof, 2008; Gioanni, 1988a) and other vertebrates (Dubois and Collewijn, 1979; Tauber and Atkin, 1968) but we did not detect such a preference for drifting responses. Overall, hummingbird drifts are roughly equal in all directions and do not exhibit a bias for a particular motion direction. A similar study with ruby-throated hummingbirds showed that head and body rotations, as well as curved flight

paths, are used to stabilize both left and right moving gratings in a cylindrical arena, and did not report any asymmetries (Ros and Biewener, 2016). Both hummingbird studies used binocular stimulation however, which may confound monocular direction preferences. Repeating an experiment with hovering hummingbirds and covering one eye of the flying subject would be important to detect directional differences.

Stationary objects are salient features for guiding flight. Tethered flies in a closed loop virtual environment will 'steer' to fixate a bar in front of them (Reichardt and Wenking, 1969). Stabilizing a stationary feature in the fovea or another portion of the visual field also offers a potential mechanism for holding station during hovering. Our flight arena was not fully immersive and included multiple stationary features including a projected image with distinct edges in the lateral field, an open ceiling with cameras, a perch, and a single feeder. During docked feeding, the clear plastic feeder (filled with clear liquid) could subtend an angle up to 26 degrees, although this feature occupies much less of the visual field during look-ups. Despite the presence of numerous stationary features – such as the feeder – visual motion consistently elicited a drift in position. This suggests that, unlike in other studies where visually-guided behavior was similar with grating and natural scene manipulations (Baird and Dacke, 2012), the visual motion stimuli used here may be especially strong compared to moving backgrounds that occur naturally.

In a natural environment, visual motion is unlikely to be coherent and unidirectional for a stationary observer like a hovering hummingbird. Motion in the visual environment also does not have to fall along perpendicular global axes, which we used in these experiments. Our results show that hovering hummingbirds drift to generally match visual motion direction, but do hummingbirds also change drift direction for small changes in the direction of the motion? In

addition, natural scenes would likely have moving and stationary components, so we predict that weakening the immersive background motion with stationary patterns would provide hummingbirds with a stable reference that could rescue normal, stationary hovering. How these results translate to flight in more natural settings remains to be investigated, but it is clear that unidirectional visual motion has a strong influence on hovering behavior.

The brains of hovering birds exhibit specialization for processing unidirectional visual motion. The accessory optic system of birds encodes optic flow produced when an observer moves relative to their environment. Its neurons have wide receptive fields and are directionally biased (Winterson and Brauth, 1985). One of the key accessory optic system nuclei is the lentiformis mesencephali, and it is hypertrophied in all birds capable of hovering, including transient hovering (Iwaniuk and Wylie, 2007). Hummingbirds have the largest lentiformis mesencephali relative to all other birds, which led Iwaniuk and Wylie to propose that directionally-selective responses are a key adaptation for controlling hovering flight (Iwaniuk and Wylie, 2007). Our behavioral experiments lend further support to this hypothesis, and hypertrophy of the lentiformis mesencephali also offers a potential mechanism for the heightened multi-directional motion sensitivity we observed.

The use of directionally selective neurons in hovering position control represents a subset of the neural mechanisms required for the flight repertoire of hummingbirds. Visual processing and integration with other sensory information has not been studied during flight in a hummingbird or in any other bird. However, evidence from fictive flight preparations of other birds and insects indicates that they can change how senses are integrated and that different neuron populations are emphasized in different behaviors. An example of variable sensory integration comes from restrained pigeons that exhibit different head stabilization reflexes and

tail responses to visual and vestibular perturbations during simulated flight than during resting conditions (Bilo and Bilo, 1978; McArthur and Dickman, 2011). At the level of sensory neurons, cells of the avian accessory optic system are divided into populations that differ in maximum sensitivity to either fast or slow motion (Winship et al., 2005; Winterson and Brauth, 1985; Wylie and Crowder, 2000). The functional roles and the relative abundance and distribution of fast and slow cells have not been described in any bird. This is in contrast to the directionally selective visual neurons of insects, which have been studied in several species and behavioral contexts (Borst et al., 2010; Taylor and Krapp, 2007). A comparative study with ten species of insects revealed that the lobula plate neurons of hovering insects are maximally sensitive to low temporal frequencies of sine wave gratings whereas the equivalent cells in fast-forward flying insects are maximally sensitive to high speeds (O'Carroll et al., 1996). Combining free flight experiments that manipulate sensory information with neuroscience approaches to understand the underlying cell populations and their responses represents an exciting new direction for avian research.

Development of virtual reality approaches with both tethered preparations and in free flight arenas has been essential for decades of research on the visual guidance of insect flight (Borst et al., 2010; Srinivasan et al., 1999; Taylor and Krapp, 2007). Similar to the hummingbirds, hovering insects have previously been shown to exhibit sensitivity to the direction (Kelber and Zeil, 1997; Kern and Varjú, 1998) and orientation (Tammero and Dickinson, 2002a) of visual motion during flight behavior. Our study joins a handful of recent avian studies that highlight the convergence between visual guidance strategies for flight in insects and birds (Bhagavatula et al., 2011; Collett and Land, 1975; Eckmeier et al., 2008; Srinivasan et al., 1991) and also suggest potential similarities in neural specialization to match flight styles. The ability to study the visual motion detection in flying hummingbirds now provides an opportunity to examine how large populations of visual neurons are used to guide behavior. We suggest that future research on the properties of the additional cells in the hummingbird accessory optic system may yield novel insight into the evolution of flight in birds.

## Figures



#### Figure 1. Anna's hummingbirds respond to moving visual patterns during free flight

**hovering.** A back-projection screen allows images to be projected onto the wall of a cylindrical flight arena with a feeder at the center of the screen. A three camera tracking system determines the head position of a freely flying hummingbird (A). The 3D traces of head position (B) are then separated into motion along three axes: forward-backward (x), left-right (y), and down-up (z). With a stationary background the bird is stationary during docked feeding (blue region) and undocked look-ups (red) near the feeder (C), but a looming spiral pattern changes the bird's position stability during both hovering phases (D). By isolating the responses during the look-up portions (red and maroon in C,D respectively) of the free flight recording we show that visual motion produces a matched destabilization response in an individual hummingbird (E).



**Figure 2.** Rotating spirals and moving gratings caused hummingbirds to lose positional stability in the orientation of the motion but stationary patterns did not affect hovering. Two motion treatments and one no-motion treatment were conducted for each of three black-and-white patterns (A): spiral (red), vertical grating (blue), and horizontal grating (green). The look-up drifts for all hummingbirds (n=8) are plotted by stimulus pattern with drift distance along the x-, y-, and z-axes shown in rows B-D respectively. Means of all drifts for a single stimulus treatment are shown in both a top view (E) and a side view (F) to illustrate the directional matching of response to stimulus motion. Almost no directional drifting occurs with no-motion treatments.


**Figure 3. Representative traces of hummingbird movement demonstrate how docked feeding and undocked look-ups differ depending upon spiral motion.** Each feeding bout is represented by three traces corresponding to the x-, y-, and z-axes. Positions are shown relative to the feeder, which is placed at approximately 0 in all directions. In the presence of looming motion (A), an individual hummingbird approached and docked at the feeder, and oscillated during feeding at less than 1 cm amplitude on the x-axis. Periodic look-ups are evident where the bird flew backward (increasing x) and upward (increasing z) with these look-ups also exhibiting backward drifts on the x-axis but little drift in the other axes. Look-ups are interspersed with short feeding events. In the presence of a non-moving spiral (B) there were no oscillations during docked feeding or drifts during look-ups. In the presence of receding motion (C), there were no oscillations during docked feeding because the feeder physically restricts movement in the predicted direction. During undocked look-ups however, hummingbird drifted forwards (decreasing x) as predicted. All representative traces (Figs. 3-5) are from the same individual on the same day.



**Figure 4. Representative traces of hummingbird movement demonstrate how docked feeding and undocked look-ups differ depending on motion of a vertical grating.** In the presence of motion to the right (A), hummingbirds drifted to the right (increasing y) during undocked look-ups with little drifting in the other axes. In the presence of a non-moving vertical grating (B) there were no drifts during look-ups. In the presence of motion to the left (C), hummingbird drifted to the left (decreasing y) as predicted. There was little motion observed during docked feeding because of the physical restriction imposed by the feeder. All other details as in figure 3.



**Figure 5. Representative traces of hummingbird movement demonstrate how docked feeding and undocked look-ups differ depending upon motion of a horizontal grating.** In the presence of upward motion (A), hummingbirds drifted upward (increasing z) during undocked look-ups with little drifting in the other axes. In the presence of a non-moving horizontal grating (B) there were no drifts during look-ups. In the presence of downward motion (C), hummingbird drifted downward (decreasing z) as predicted. There was little motion observed during docked feeding because of the physical restriction imposed by the feeder. All other details as in figure 3.



Figure 6. Pairwise comparisons for movement along the x-axis demonstrate that looming and receding motion elicit strong backward and forward flight responses. Each panel presents the post-hoc test associated with a linear mixed effects model. The upper panels correspond to post-hoc analyses for backward movement during undocked look-ups (left) and docked feeding (right). The lower panels correspond to post-hoc analyses for forward movement. Each panel contains 36 pairwise comparisons derived from nine stimulus levels. The stimulus levels (1-9) are assigned in the same order as the panels in figures 3-5. For example, the top comparison (2-1) is the difference between a no motion spiral and a looming spiral. Black circles and brackets indicate the effect estimate and 95% confidence interval for comparisons where the stimulus level is not predicted to affect that type of movement. Colored circles and brackets indicate the same for comparisons where the stimulus is predicted to affect that type of movement. The colors correspond to movement direction in all figures. Looming produces significantly more backward motion during both undocked look-up and docked feeding segments. Forward motion during undocked look-ups is similarly much higher in the receding spiral treatment. During docked feeding, the oscillations produced by looming are also evident. For all other stimulus levels, movement in the predicted direction is physically blocked by docking with the feeder.



**Figure 7. Pairwise comparisons for movement along the y-axis demonstrate that right and left visual motion elicit strong right and left flight responses.** The upper panels correspond to post-hoc analyses for movement to the right during undocked look-ups (left) and docked feeding (right). The lower panels correspond to post-hoc analyses for movement to the left. Moving a vertical grating to the right produces significantly more flight to the right during look-ups compared to other stimulus levels. Moving a vertical grating to the left produces significantly more flight to the left during look-ups compared to other stimulus levels. During docked feeding (right panels), the effect estimates were all close to zero, even for the oscillations produced by looming visual motion (maroon). All other details as in figure 6.



Figure 8. Pairwise comparisons for movement along the z-axis demonstrate that upward and downward visual motion elicit strong upward and downward flight responses. The upper panels correspond to post-hoc analyses for upward movement during undocked look-ups (left) and docked feeding (right). The lower panels correspond to post-hoc analyses for downward movement. Moving a horizontal grating upward produces significantly more upward flight during look-ups compared to other stimulus levels. Moving a horizontal grating downward produces significantly more downward flight during look-ups compared to other stimulus levels. During docked feeding (right panels), the effect estimates were all close to zero, even for the oscillations produced by looming visual motion (maroon). All other details as in figure 6.



# **Figure 9. Frequency of look-ups and feeding duration are similar among different stimulus treatments.** The frequency of look-up events (A) does not vary consistently across the nine stimulus treatments (B). For feeding duration (C), there is a significant difference when receding spiral trials are compared to most other stimulus treatments. Other comparisons are not significant. Each column contains the data for a single background stimulus treatment and is colored to match figures 1-8. The two trials for each individual hummingbird within a single stimulus column are indicated by symbols and are slightly separated along the x-axis.

# CHAPTER 2 – HOVERING HUMMINGBIRDS RESPOND TO SMALL CHANGES IN BACKGROUND LOOMING MOTION

### Introduction

Animals moving through their environment can use vision as a source of information about posture and position (Gibson, 1950, 1954; Lee, 1980). The motion of visual features across the retina is called optic flow, and is an important signal of motion, either of the environment relative to the observer, or the opposite, the motion of the observer relative to the environment. When the observer moves, the motion is termed self-motion, and the optic flow produced on the retina has a characteristic pattern depending on the type of motion. For example, body rotation looks different than moving forward along a straight path. Many animals exhibit postural or position changes in response to visual motion created in laboratory settings by moving background patterns (Kern and Varjú, 1998; Ros and Biewener, 2016; Shaw and Tucker, 1965; Soechting and Berthoz, 1979; Zeil and Wittmann, 1989). The motion stimuli used in these behavioral studies are traditionally immersive, high-contrast, and coherent, which brings into question their relevance for animals behaving in a complex natural landscape.

Natural scenes can be relatively simple, but can also be complex mosaics with patches of varying color, brightness, shape, motion direction, motion speed, and texture. In experiments including treatments with natural scenes, natural light conditions, and three-dimensional stimuli, bumblebees in complex environments exhibited similar flight responses to those in traditional black-and-white experimental environments (Baird and Dacke, 2012). Motion detecting neurons in hoverflies were shown to respond to motion of natural scenes across a range of scene

compositions and contrasts (Straw et al., 2008). These studies show that both behavior control using visual motion and underlying motion detection are comparable in laboratory and naturalistic settings. In contrast to insect systems where visual control of flight paradigms are well established, avian flight control studies are just beginning to identify important visual cues and have not explored more complex visual scenes.

Hummingbirds in natural and laboratory settings are able to precisely control their body position during hovering. We have shown that large, coherent, black-and-white pattern motion change hovering in Anna's hummingbirds, and that the resulting drift responses match the stimulus motion direction (Chapter 1). A more recent study using a cylindrical arena with full 360 degree stimulus projection similarly found that left and right grating motion elicited head and body rotations in hovering ruby-throated hummingbirds (Ros and Biewener, 2016). We have also shown that hummingbirds respond to looming motion with backward drifts even when they are feeding (Chapter 1; Goller, 2011). The coherent motion of the visual environment completely changes hummingbird hovering in the laboratory, but how does hummingbird sensitivity to motion translate to more complex visual scenarios?

We predicted that hummingbird responses were strong primarily because the stimuli projected onto flight arena walls in previous experiments were large, coherent, and high-contrast (Chapter1; Goller, 2011; Ros and Biewener, 2016). In addition, our experiments tested three perpendicular axes of motion, requiring a different pattern for each axis, which only enhanced the artificial nature of the background motions and their different directions. To investigate hummingbird sensitivity to visual motion during hovering with less contrasting treatments, we focused on the backward drifting response to a looming spiral because hummingbirds could respond during both hovering in space and at the feeder. It was important to measure many

behavioral responses in order to detect smaller adjustments that are more likely characteristic of hummingbirds making position adjustments to small changes in their visual environment.

Are hovering hummingbirds sensitive to small changes in motion direction? The direction of expansion for a looming spiral pattern is determined by the position of the spiral center. Without changing patterns, visual motion direction could be changed slightly to no longer align with the forward-backward axis of a feeding hummingbird, creating a more natural scenario where motion was not restricted to major global axes. We investigated hummingbird sensitivity to small changes in visual motion direction by moving the spiral center up, down, left, or right and measuring changes in the backward drift response. Drift responses were predicted to be strong and tuned to the motion direction because the strength of the stimulus remained unchanged. We followed with an experiment designed to test the role of visual motion strength on hovering position stability.

Does a complex visual background, with stationary and moving patterns, rescue hovering position stability? To investigate this question we created a composite stimulus where a circular mask with varying radius, and containing one pattern, was centered in front of a different pattern. A checkerboard and looming spiral were used for the paired patterns and could be alternately placed in either the mask or the background position. We hypothesized that the checkerboard would provide enough stationary contrast to the moving pattern to allow the hovering hummingbird to maintain a stationary position in space. The aim was to determine the relative amount of stationary pattern required for a hummingbird to regain hovering stability when the background contained visual motion.

### Methods

Animal model: Experimental subjects were eight male Anna's hummingbirds, *Calypte anna*, that were caught on the campus of the University of British Columbia (UBC, caught November 5, 2012-October 29, 2013). Hummingbirds were individually housed in 0.61 x 0.61 x 0.91 m cages and fed *ad libitum* sugar (15% by weight) or Nektar-Plus (Nekton, 13% by weight) solution. Individuals were allowed to acclimate to captivity for three days, and were then trained in the experimental chamber with a feeding schedule. The feeder, filled with sugar solution, was closed to prevent feeding between experimental trials, and opened at intervals of 20 minutes during training and the experiments. When the feeder was opened, birds were allowed to feed until they departed. Restricting food between feeding was important to increase the time birds spent at the feeder. All experiments were performed with approval of the University of British Columbia Animal Care Committee.

**Experimental rig:** The experimental chamber was a large clear acrylic cylinder (Fig. 10A; 0.70 m diameter, 0.61 m height). A 0.91 m section of the wall (41.4% of circumference, 100% of height) was covered on the outside by a frosted window coating (wallpaperforwindows.com) allowing back projection onto the cylinder. We used a Canon REALiS SX80 Mark II LCoS (Liquid Crystal on Silicon) projector (3000 lumen lamp, 1400x1050 SXGA, 60Hz). Visual stimuli were generated and controlled using custom scripts in VisionEgg (Straw, 2008).

At the center of the projection screen (approximately 180 degrees horizontally and 102 degrees vertically) was a small hole where a clear plastic feeder was attached such that it extended 0.175 m into the chamber. The ceiling of the chamber was nylon mesh with holes for the lenses (Computar H2Z0414C-MP) of three cameras (Prosilica GigE680, Allied Vision Technologies). These three cameras were used to automatically track a painted white spot on the

top of the bird's head using Flydra 3D tracking software (Straw et al., 2011). Filming was conducted at 100 frames per second (fps). The tracked flight trajectories were converted into 3D (x,y,z) coordinates (Fig. 10B) and exported for further analysis using custom scripts in Matlab (Mathworks R2012a).

**Stimulus description:** The spiral pattern was produced using Matlab to draw a four-armed 10 degree logarithmic spiral. Areas between the four spiraling lines were filled with black and white in alternating fashion. The spiral pattern was rotated in clockwise direction at a rotation frequency of 0.5Hz. Clockwise rotation (looming) at this frequency corresponds to a time-to-collision ( $\tau$ ) value of 1.86 seconds (Martinoya and Delius, 1990). We tested the luminance of the spiral projected with the Canon LCoS projector. White spiral segments ranged from 316 to 160 cd/m<sup>2</sup> from the center of the spiral to the periphery. Black segments measured 87 cd/m<sup>2</sup> in the center and 35 cd/m<sup>2</sup> at the periphery. For pattern combinations, a stationary black and white checkerboard pattern with 0.5 cm squares was used. In addition the width of the projection screen was decreased to 120 degrees horizontally and 100 degrees vertically in an attempt to make a more square projection area to facilitate relative area calculations.

**Experiment protocol:** Both experiments relied on filming during a prolonged feeding bout and trials were conducted every 20 minutes. Each experiment lasted a single day. Any feeding flight where the hummingbird consumed less than 0.25 milliliters of sugar solution was discarded and the trial was repeated. Access to food was restricted by removing the feeder between trials and any moving stimulus patterns were stopped. Trials stopped when a bird finished feeding (left the camera views) or after two minutes without a bird approaching the feeder. Restricting the timing of feeding bouts and only providing a 12% by weight sucrose solution increased the feeding duration but also necessitated training before experiments could start.

The experiment testing the response to an offset spiral center had five treatments where the center of a looming spiral was moved left, right, up, down or remained centered in the background. The spiral center was moved by approximately 0.105 m or 30 degrees (from the viewpoint of a docked hummingbird) in all directions. Because the spiral pattern was larger than the projection area, the entire screen was always filled with spiral pattern. Each stimulus was repeated twice and the overall sequence was randomized for six individuals in total.

The experiment testing the response to stationary pattern combined with a looming spiral used a square projected image (288 x 288 pixels) partitioned into a background image (either checkerboard or spiral) and a circular image with variable radius overlaid in the center (opposite pattern). The spiral could be stationary or rotating. By changing the size of the central circular image, we tested how sensitive the birds were to different amounts of visible looming. By alternating the pattern in the middle, we changed the location of the looming stimulus, either in the center of the image or at the periphery. Preliminary experiments suggested that birds exhibited less response at small amounts of visible spiral so the first four birds were tested with small central spirals and large central checkerboards (limited spiral visible at the periphery). Six different radius treatments were used for the central looming spiral: 80, 88, 96, 104, 112, 120 pixels corresponding to 24.24, 29.33, 34.91, 40.97, 47.51, and 54.54% of the projected image respectively. A large series of radii were used for the central checkerboard to obscure most of the spiral as well, with central checkerboards with radius 144, 136, 128, 120, 112, and 104 pixels yielding spiral percentages of 21.46, 29.94, 37.94, 45.46, 52.49, and 59.03 respectively. Nomotion trials were also conducted for the 96 pixel radius circle with both a central spiral and a central checkerboard. In the first phase of data collection, 14 randomly ordered trials were conducted for each individual and no trials were duplicated.

We later decided to conduct the treatments with greater percentages of visible looming spiral and tested an additional four hummingbirds with the large radii (listed above) used for central looming spirals (144-104 pixels: 78.54, 70.06, 62.06, 54.54, 47.51, 40.97%) and the small radii used for central checkerboard patterns (80-120 pixels: 75.76, 70.67, 65.09, 59.03, 52.49, 45.46%). Again, no-motion trials were conducted for a total of 14 different stimulus treatments during the second phase of data collection. Thus, there were 26 different stimulus treatments for this experiment.

**Analysis - Response to offset spiral center:** To examine how hummingbirds responded to the position of looming visual motion, we analyzed their movements in a spherical coordinate system. Every position was defined by a radius and two angles: azimuth and elevation (Fig. 10C). Movements towards the feeder were defined as negative, and movements away from the feeder were defined as positive. We first calculated the derivative of the radius and then extracted sequences of backward flight that were at least 0.25 seconds in duration. By taking the start and end points of these segments, we calculated a single vector to describe the backward motion in three dimensions. The azimuth and elevation angles of the vector, which describe flight direction, are presented in figure 11. The radius (magnitude) of the backward flight segments is presented in figure 12.

To evaluate whether hummingbirds responded to the change in center of expansion, angles and radii were analyzed using linear mixed effects models in R (Pinheiro et al., 2016; R Core Team, 2015). Stimulus was treated as a fixed effect with individual bird as a random effect. Because the 10 trials per individual (two each of center, left, right, up, and down) were conducted in random order and we were not specifically interested in an overall response magnitude (radius), these models do not account for a possible response change over the course

of the experiment within individual. Post-hoc testing to differentiate between the stimulus treatments was done with Tukey tests using the multcomp package (Hothorn et al., 2008).

**Analysis - Response to combined stationary pattern and looming spiral:** A bird's position and velocity were used to define two types of hovering behavior, which could each appear multiple times within a single feeding bout. *Docked feeding* segments are defined by the bird being within physical reach of the front of the feeder and having a net velocity less than 0.050 meters per second. *Undocked look-up* segments are defined by the bird not being within physical reach of the front of the feeder but still having a net velocity less than 0.050 meters per second. Only segments that lasted at least 0.25 seconds were included in analyses. To examine responses to looming motion in the presence of stationary features, we extracted only the docked feeding segments.

For each segment of docked feeding we calculated the average movement along the forward-backward axis (x-axis in Chapter 1). Forward movement was defined as negative and backward was positive. We first calculated the derivative of the bird's position, then sorted the derivative by motion direction (positive vs. negative values) and all values pertaining to a single motion direction were summed to get a total distance traveled in each direction (purple highlight, Fig. 10B). We next normalized the summed movement by dividing it by the duration of the segment. Thus, forward and backward movement are represented as movement (in centimeters) normalized to one second of flight (Fig. 10C).

Behavior analysis focused on responses during docked feeding flight because undocked look-ups occurred with unpredictable frequency. The hummingbirds exhibit individual and dayto-day variation in how regularly undocked look-ups are performed but docked feeding is consistent and predictable. The motion in the backward direction was normalized for the length

of the flight segment as above. The visual stimulus was described as a percentage of looming spiral pattern and stationary pattern, such that a totally stationary stimulus would have 0% looming and 100% stationary, but a looming spiral without a stationary pattern would be 100% and 0% looming and stationary respectively. The backward response was also related to the order of the experimental trials because previous work had shown a decrease in response magnitude over time (Goller, 2011). These data describing response, stimulus composition, and trial order (1-14) were analyzed using non-linear mixed models in R (Pinheiro et al., 2016). Linear and exponential models did not fit the data well, so we used a logistic function. Model parameters were estimated using individual bird as a random effect and then compared to models where trial order was additionally included as a fixed effect. These curves had the formula:

$$f(x) = i + \frac{a}{(1 + e^{-s \cdot (x - c)})}$$

where a is the upper asymptote, s is a rate term, c is the center, and i is the lower asymptote. The terms were estimated by fitting the model for data with varying x, percentage of spiral or stationary pattern.

### Results

### Hummingbirds respond to small changes in looming spiral orientation

As the hummingbirds attempt to stabilize visual motion, how precisely do they match small changes in the direction of the stimulus? To address this question, we shifted the center of the spiral left, right, up, and down with respect to the feeder to determine if the angular orientation of the flight response was coupled to the center of expansion in the looming stimulus (Fig. 11A-B). Shifts in spiral position caused hummingbirds to shift the angle of their backward responses for both the azimuth ( $\theta$ ) angle (Fig. 11C;  $F_{4,1197} = 213.4$ , P < 0.0001) and the elevation ( $\alpha$ ) angle (Fig. 11D;  $F_{4,1197} = 170.7$ , P < 0.0001). The length of the backward response drifts (radius) was also affected by shifting the spiral center (Fig. 12;  $F_{4,1197} = 6.805$ , P < 0.0001).

To compare the effects of the different offset positions on the angle and radius measures, post-hoc Tukey tests were performed. Left and right offsets of the spiral center caused significant deviations in azimuth angle compared to centered (left: estimate = 17.133, P < 0.001; right: estimate = -24.676, P < 0.001) but there was no azimuth difference between centered and the up and down offsets (up: estimate = 1.020, P = 0.961; down: estimate = -3.070, P = 0.184). For elevation angle the up and down offsets were predicted to affect the bird response. All directions were significantly different from centered (up estimate = -10.796, down estimate = 25.747, left estimate = 7.509, right estimate = 7.006, all P < 0.0001). The left and right treatments were, however, significantly different from the up and down treatments (estimates = -17.802 to -18.741, all P < 0.0001), but not different from each other (estimate = -0.503, P = 0.997). Shifting the center of the spiral by 30 degrees caused the radius of backwards drifts to get shorter (estimates = -0.353 to -0.556; P < 0.0173) but the different offset directions were not different from each other (estimates = -0.203 to 0.0372; P > 0.395).

How close did the hummingbirds come to matching the stimulus offset? The magnitude of the angular response for elevation in the up-down offset trials and azimuth in the left-right offset trials was similar (estimate = -2.235, P = 0.052). Both were significantly different from the maximum angular response (maximum of both angles measured for each drift) to centered spiral position (elev. estimate = 6.131, azim. estimate = 8.367, all P < 0.0001). With offsets of 30 degrees, a 30 degree response was predicted for exact matching. Mean individual responses to a left shift ranged from 16.56 to 33.44 degrees, and -24.46 to -3.57 degrees for a right shift. Up and down elicited vertical responses of -22.57 to -5.23 degrees and 17.26 to 35.58 degrees respectively.

In addition to analyzing every drift event, statistics were also calculated based on mean individual responses for the six hummingbirds to each of five treatments. The mean analysis suggests that there was no significant effect of stimulus on response radius ( $F_{4,20} = 2.260$ , P = 0.0987). Up and down shifts had a significant effect on elevation angle (up estimate = -12.127, down estimate = 26.576, all P < 0.001) and none on azimuth (up estimate = 1.009, down estimate = -2.657, all P > 0.89). Left and right shifts had an effect on azimuth only (left estimate = 15.401, right estimate = -23.632, all P < 0.0001), and none on elevation angle (left estimate = 7.079, right estimate = 6.884, all P > 0.064). Overall, this analysis showed that on average, individuals are drifting to match the stimulus direction.

### Hummingbirds respond to looming motion even when portions of the spiral are covered

We have demonstrated that hovering hummingbirds consistently lose position in response to moving visual patterns (Chapter 1) using stimuli that cover a large portion of their frontal visual field. In addition, the previous experiment showed that small changes in the position of the center of a looming spiral pattern change the angle of backward drift responses. The second experiment tests the hypothesis that hummingbirds will maintain stable position, in the presence of visual motion, if there are prominent stationary features in their visual field, especially if these cover the center of the spiral pattern. We combined looming visual motion from the spiral with a prominent stationary pattern, a black-and-white checkerboard. Because the center of the moving spiral pattern appears to be especially important, we tested two configurations: 1) the spiral obscuring the center of the checkerboard, and 2) the checkerboard obscuring the center of the

spiral. For each configuration, the backward response was measured over a range of relative spiral and checkerboard sizes.

When the looming spiral obscured the center of a stationary checkerboard, the backward response increased monotonically with the increase in looming area (Fig. 13A). Surprisingly, even though hummingbirds respond to the position of the center of a spiral (Fig. 11), the same backward response trend was observed when the checkerboard obscured the center of the spiral (Fig. 13B). The relationship between the backward response and the area of the central pattern was analyzed using non-linear mixed models, with individual bird as a random effect, estimating four parameters: a = upper asymptote, s = rate term, c = center, and i = lower asymptote. Curves without a lower asymptote (*i*) were also fitted but these did not improve the model for increased looming (ANOVA, AIC<sub>no i</sub> = -152.06, AIC<sub>with i</sub> = -150.99, P = 0.112) and were significantly worse for increasing stationary background (ANOVA, AIC<sub>no i</sub> = -84.48, AIC<sub>with i</sub> = -108.42, P < 0.0001).

A model that included trial order for the asymptote term was the best model for the increasing central spiral response, and a slight but significant improvement over the second best model which did not include trial order (ANOVA, AIC<sub>no order</sub> = -149.03, AIC<sub>a-order</sub> = -150.99, P < 0.0467) suggesting that there was a decrease in response magnitude over time. Similarly for increasing central checkerboard, the asymptote with order version was the best model (ANOVA, AIC<sub>i,s-order</sub> = -99.48, AIC<sub>a-order</sub> = -108.42, P = 0.0084). The parameter estimates for the best-fit models are shown in Table 1 and the curves are plotted with the raw data in figure 13 with trial order = 0. If trial order were included in the plot, there would be 14 curves, one for each permutation of *a*, and shifted by their different upper asymptote.

The sigmoidal functions were used to predict a response with 100% looming spiral motion and plotted against the average backward response for the same individual birds from experiments in Chapter 1. The average response to looming motion was to drift backward 0.79 cm every second of feeding. The model based on an experiment with a pattern that covers less of the visual field still predicted this saturated response. The model for increasing central spiral predicted 0.68 cm (0.60 for highest trial order effect) and the model for a central checkerboard series predicted 0.95 cm per second of feeding (0.58 for highest trial order effect), within the spread of the individual responses (Fig. 13). Trial order had a 4.3 fold greater effect on the peripheral spiral series than the central spiral series. Models would predict that the response would reach 50% of max when a central spiral was 52.72% of the projected image, or 53.78% on the periphery of the image.

### Discussion

Hummingbirds hovering in flight arenas with immersive, moving black-and-white patterns on the walls, lose positional stability and track visual motion with head and body rotations and drifts (Chapter 1; Goller, 2011; Ros and Biewener, 2016). These experiments show hummingbird sensitivity to motion, but use large, coherent visual stimuli and large contrasts between motion directions. Such strong visual signals are unlikely to occur during hovering in natural settings, so we tested more subtle changes in a looming spiral stimulus to investigate whether hummingbirds would respond to small changes in motion direction or regain positional stability with stationary patterns. Our experiments show that hummingbirds are sensitive to relatively small changes (30 degrees) in left-right and up-down direction of optic flow. Their sensitivity to motion is further shown by their continued destabilization, even when stationary

visual features are present and layered with the motion stimulus. The response increases in sigmoidal fashion as the relative area of moving versus stationary pattern area in the background image increases, both when the center of the spiral is visible, and when it is obscured.

A rotating spiral pattern creates the illusion of continuous expansion or contraction depending on the direction the pattern is rotated. Visual expansion is characterized by an origin, the spiral center, from which motion is directed radially outward and toward the observer (Martinoya and Delius, 1990). Expanding visual motion is characteristic of an approaching visual feature and of forward observer motion (Gibson, 1954; Lee, 1980). When expansion is linked with an object or feature, it can signal upcoming collision and requires action on the part of the observer, who has to decide whether to avoid the object (Tammero and Dickinson, 2002a) or interact with it, possibly by landing (Braitenberg and Ferretti, 1966; Coggshall, 1972; Lee et al., 1993) or docking (Lee et al., 1991). The slow expansion cues used in these experiments, create motion characteristic of 1.86 seconds to collision, which does not appear to alarm hummingbirds, and instead produces slow backward drifts that could serve to stabilize the expansion cue. In addition, hummingbirds exhibit similar drifting response to visual motion in directions other than looming (Chapter 1; Goller and Altshuler, 2014), which suggests that the visual background motions used in these experiments are more likely perceived as signals of observer motion than object motion.

Visual expansion around a center is motion characteristic of an observer moving forward. To stabilize the motion, the observer would have to move backward, as seen in the hummingbirds (Chapters 1-2). When the center of expansion is moved left, right, up, or down, the direction of the illusory observer motion reorients toward the new center of expansion, therefore requiring a change in the angle of the compensatory backward response. In addition to

the offset in the stimulus image, our projection surface was the wall of a cylinder so there was also some displacement along the curvature of the wall for the left and right spiral positions. The large radius of the cylinder (0.35 meter radius), as well as the small 10.5 centimeter displacement of the spiral center along the 2.2 meter circumferential midline, make this effect minimal. There was no significant difference between the magnitudes of horizontal response angle to left-right offsets and the vertical response angle to up-down offsets, suggesting the curvature did not increase the apparent direction change significantly. If anything, birds more closely matched left and down shifted spirals than those shifted up and to the right. The horizontally shifted spiral also caused a response in the elevation angle, albeit a small one that did not show up in individual mean analysis. Perhaps adding a left or right component to the backward drift necessitates an upward component as well. In retrospect, it would have been interesting to include intermediate offsets, such as upper left, or lower right, to determine how closely hummingbirds matched these combinations.

Vection is a phenomenon where subjects perceive or feel that they are moving (Palmisano et al., 2015), and visual stimuli that induce vection have been extensively studied in humans. We do not know if hummingbirds seeing our expanding stimulus perceive themselves to be moving forward, as required of this definition. Hummingbirds need not 'perceive self-motion' to exhibit compensatory behaviors. Postural sway in humans can occur at motion thresholds lower than those required for inducing illusory self-motion (Previc and Mullen, 1990; Stoffregen, 1985). Instead of claiming that these hummingbirds are experiencing vection, we reference vection literature as a context for discussion of properties of the visual stimuli because these same features may be generally important for vertebrate visual processing to direct compensatory behaviors to visual motion.

Shrinking the size of the projection screen to approximate a square surface and then layering different patterns, may have enhanced the response to motion by introducing a depth cue. An external frame, like looking through a window, increases vection (Telford and Frost, 1993). A central mask, however, was reported to have limited effect on a laminar flow pattern inducing vection. Without a mask, vection was induced 81% of the time, while a 54 degree circular mask on a 96 degree by 78 degree wide angle screen reduced the illusion of self-motion to 62% (Telford and Frost, 1993). Our non-linear mixed effects model, fit to hummingbird response data for a looming spiral with increasing sizes of a central checkerboard mask, predicts a maximum backward response of 0.953. Calculating our model prediction for a circular mask with 54 degree radius (30.6%) yields 0.708 for a 26% reduction, very similar to the actual 23% reduction in vection measured by Telford and Frost. Vection induced by radial dot motion, which is more similar to the looming or expansion of the spiral, was not affected by masking the center, but decreased when masking the periphery (Telford and Frost, 1993). It is perhaps notable, that hummingbird avoidance responses were much more symmetrical for a central and peripheral mask than the perception of self-motion in human subjects. We did not test a variety of different motion types, but predict similarly strong responses for all motion directions based on our previous results (Chapter 1).

It is also important to note that it is difficult to compare central and peripheral portions of a stimulus in experiments with birds and humans. A frontal screen occupies a different portion of the visual field for a largely binocular human compared to a laterally eyed bird, like the Anna's hummingbird, which has limited binocular overlap (Martin, 2007, 2009). We found that peripheral motion is as important, if not more so, than central motion. The periphery of our projection screen was located about 50-60 degrees in each direction from a central point defined

by the feeder-bill axis. Hummingbird retinas have both a shallow temporal specialization and a more lateral fovea (Lisney et al., 2015; Wood, 1917), but in addition to responding to peripheral motion they also respond to small central spirals and shifts in spiral center. Moving the center, while it is obscured by the mask, would provide an interesting experiment to determine if the orientation of expansion could as easily be perceived from just peripheral motion.

Hummingbirds are highly sensitive to visual motion and motion direction during hovering, and drift in the direction of the visual motion. Visual expansion from a rotating spiral pattern causes hummingbirds to lose the ability to hold a stationary position during both free hovering in space and hovering while feeding. Non-expansion visual motion could only be studied in undocked hovering birds using visual tracking because docking with a rigid feeder prevented hummingbirds from moving left, right, up, down, and forward. Yet experimental attempts to rescue normal hovering behavior by covering moving patterns with stationary ones suggest that any expanding visual motion is sufficient to cause feeding hummingbirds to change body position. We suggest that tactile feedback from bill contact with the feeder can serve as a strong stabilizing signal, and that fusion of tactile and visual information will be an important part of docked hovering position control.

## Figures



Figure 10. The position of hummingbirds hovering near a projection screen was measured in 3-dimensions. The acrylic flight chamber has a back-projection screen on one side with a feeder centered in the screen (A). Hummingbird position was tracked with three synchronized cameras filming from above at 100 fps. Computer controlled stimuli could be projected with an external projector. Position of the hummingbird (B) was calculated using the multiple camera views and broken into forward-backward position (x), left-right position (y), and down-up position (z). Taking a derivative of the x-position allowed us to highlight the flight segments where the bird moved backwards (derivative > 0) for both feeding (purple) and look-ups (red). Two different analyses were conducted for these experiments (C, gray inset). In the first, backward drifts at least 0.25s in duration were described using a spherical coordinate system: a radius term, a horizontal (azimuthal,  $\theta$ ) angle, and a vertical (elevation,  $\alpha$ ) angle. The second analysis is an overall backward response during feeding, where all backward motions during a feed are summed and normalized to the duration of the feeding segment.



Figure 11. Hummingbirds shift the orientation of their backward flight to match offsets in the position of the looming spiral. The angular positions of the stimulus (s) and response (r) were defined using two mirrored spherical coordinate systems (A). We moved the center of the spiral by 30 degrees in either the horizontal (azimuthal angle,  $\theta_s$ ) or vertical (elevation angle,  $\alpha_s$ ) plane (B). Left and right spiral positions elicited matched changes in backward flight in the horizontal plane ( $\theta_t$ ) and limited change in the vertical plane ( $\alpha_t$ ) (C,D). The opposite is true for spiral center offsets above or below the center. The thick black lines in the offset conditions indicate the magnitude of a perfectly matched response at 30 degrees. Each column of points within the treatments represents the backward drifts for a single individual (n = 6) with the overall mean of all individuals shown by the horizontal bar.



**Figure 12. Moving the looming spiral center left, right, up, or down causes the radius of the flight response to decrease relative to a centered spiral.** Compared to the centered condition, hummingbirds exhibit significantly shorter backward avoidance flights when the center of the looming spiral is offset by 30 degrees. None of the offset conditions were significantly different from each other. All other details as in figure 11, except no predictions for effect magnitude could be made (no thick black line).



Figure 13. Even with a prominent stationary pattern, a looming spiral produces the characteristic avoidance response, which increases with an increased proportion of looming spiral in the projected image. The backward responses to looming motion, when it is centrally embedded in a checkerboard background, are on the left (A, green). Responses to a stationary image, a checkerboard, in the middle of a looming spiral, are on the right (B, blue). The dashed portions of the green and blue lines are the fitted sigmoidal model projection for images with >80% looming spiral pattern and 100% spiral response is shown for reference using data from previous experiments (Chapter 1, with a larger projected image). The two controls (0% in A and 100% in B) were performed with an image that included a stationary checkerboard and a non-rotating spiral. Symbols indicate individual birds (n = 8).

Table 1. Parameter estimates for series of combined patterns with either increasing		
looming spiral or increasing stationary pattern		
parameter	increasing looming spiral	increasing stationary pattern
a	0.657 – 0.00652*trial order	0.941 – 0.0280*trial order
S	0.0704	-0.0656
с	56.694	42.051
i	0.0571	0.0685
# CHAPTER 3 – HOVERING HUMMINGBIRDS ATTEMPT TO STABILIZE VISUAL MOTION DURING DOCKED FEEDING

## Introduction

Animals moving through and interacting with their environment use information from numerous sensory sources to control their behavior. Animals in motion, or attempting to stay still in a moving environment, can use vision, especially optic flow - the motion of visual features on the retina - for behavior control. A moving observer creates different optic flow patterns depending on their motion (Gibson, 1958; Lee and Lishman, 1977) and many experiments have found examples of animals using properties of different optic flow fields to control behavior. Optic flow is important for steering (Bhagavatula et al., 2011; Dakin et al., 2016; Mronz and Lehmann, 2008; Scholtyssek et al., 2014; Tammero and Dickinson, 2002a), velocity and altitude control (Baird et al., 2006; Schiffner and Srinivasan, 2015; Srinivasan et al., 1991), distance estimation (Srinivasan et al., 1997), landing (Braitenberg and Ferretti, 1966; Lee et al., 1993), docking (Lee et al., 1991), diving (Lee and Reddish, 1981), etc., and used by animals moving through water (Junger and Dahmen, 1991; Shaw and Tucker, 1965), on land (Lee & Aronson 1974), and in air (Goller and Altshuler, 2014; Kelber and Zeil, 1997; Kern and Varjú, 1998) for position or posture control. Perception of visual motion is important for guiding behavior, but other sensors also provide important information for controlling animal movement. How does non-visual sensory information change the use of optic flow to control flight?

Depending on the behavior and environmental context, the information from some senses, even vision, may not be reliable or appropriate to control a behavior. Visual motion, for example, can cause stationary human observers to perceive themselves to be in motion (Lee, 1980; Telford and Frost, 1993). Previous studies show that hummingbirds stabilize visual motion during hovering flight (Goller and Altshuler, 2014; Ros and Biewener, 2016) much like humans controlling posture. Expanding visual motion causes feeding hummingbirds to drift backward away from the feeder. Hummingbirds exhibit this backward response to expansion even in the presence of large stationary visual features that obscure the center of expansion (Goller and Altshuler, 2014). If visual references cannot help a hummingbird to remain stationary in the presence of visual motion, perhaps another sensor could?

Animals integrate information from multiple sensory sources. Fusing the information from different sensory systems to guide behavior involves not only compiling sensory information, but also constantly evaluating its reliability and appropriateness. Simple physical contact with a stable object increases stability of human posture when vision is unavailable or the visual field moves, even if the contact is not forceful enough to provide body support (Holden et al., 1994; Jeka et al., 2000; Jeka and Lackner, 1994; Oie et al., 2002). Additionally, large perturbations in visual information increase the reliance on ankle proprioception and vice versa, though not symmetrically (Hwang et al., 2014; Logan et al., 2014). Hummingbirds dock with, or insert their bills into, flowers or feeders to collect nectar, and therefore have a stable tactile reference to help them maintain body position during hover feeding. We hypothesize that hummingbirds feeding in the presence of a moving visual background would use tactile information to stabilize.

Previously, hummingbirds were shown to respond to visual motion during undocked hovering, but only backward responses could be measured for docked birds. Lateral and vertical changes in head position were restricted because the bill was inserted into a rigid feeder (Goller and Altshuler, 2014). We predicted that feeding hummingbirds would hold a stable position relative to their feeder when hovering in front of stationary backgrounds, or when the background moved laterally or vertically. We also predicted that different motion speeds, especially slow motions, could affect how tactile and visual information were integrated. To test these predictions, we designed a flight chamber with an instrumented feeder that measured the forces a bird exerted on the feeder when docked. We recorded feeder measurements for hummingbirds hovering in front of stationary and moving backgrounds to investigate three questions:

- 1) Do hummingbirds push against the feeder during hovering when the visual environment is stationary?
- 2) Do hummingbirds attempt to stabilize lateral and vertical visual motion during feeding?
- 3) Does hovering stability depend on visual motion speed?

#### Methods

Animal model: All experiments were performed with eight male Anna's hummingbirds, *Calypte anna*, caught and held on the campus of the University of British Columbia. Birds were caught between 29 October 2015 and 07 March 2016 and experiments were performed between 04 February 2016 and 17 March 2016. The hummingbirds were housed individually in 0.91 x 0.61 x 0.61 meter cages and fed *ad libitum* 15% (by weight) table sugar solution or 13% (by weight) Nektar-Plus solution (Nekton GmbH, Germany) in their home cage. Animals were collected from their home cage immediately prior to experiments, and returned immediately following experiments. All procedures were performed with approval of the University of British Columbia Animal Care Committee.

Experimental rig: The experiment chamber consisted of an acrylic cube with 0.5 x 0.5 meter wall panels (Fig. 14A). One side panel was coated with a frosted window coating (wallpaperforwindows.com) to allow back-projection with a Canon REALiS SX80 Mark II projector (3,000-lumen lamp, 1400 × 1050 SXGA, 60 Hz). The floor of the cube was partially open and covered with nylon mesh to allow downwash from the wings to escape. The cube face to the bird's right during feeding contained a door for access to the chamber. All three uncoated walls were covered with black poster paper on the inside of the chamber to prevent reflection of projected stimuli. Bird behavior was monitored at 10 frames per second using an AVT GigE680 machine vision camera (Allied Vision, Exton, PA) and the video stream was recorded during experimental trials using Streampix 7 software (NorPix, Inc., Montreal, Canada). A feeder was centered in front of the projection screen with the tip of the feeder 23 centimeters from the wall surface and filled with 0.6 milliliters of 15% by weight table sugar solution before each experimental trial.

**Feeder sensor:** The base of the small sugar reservoir was mounted on a home-built sensor that measured strain in the left-right and up-down directions. The sensor was composed of 3-D printed plastic pieces connected by four 13x5 millimeter pieces of 0.003" 18-8 stainless steel shim stock. Each small shim stock piece had a N2A-13-S071P-350/LE2 model strain gauge mounted with MBond 610 adhesive (Vishay Precision Group, Malvern, PA). These were wired such that gauges in opposite orientations were mounted on opposing halves of a single Wheatstone bridge for each axis measured (one bridge with two gauges for left-right and another for up-down). Bridges were completed with 350 Ohm resistors. Each bridge was separately powered by a 9 Volt battery and amplified in separate channels of a Brownlee Precision Model 440 four-channel amplifier (NeuroPhase LLC, Santa Clara, CA). Signal from the sensor was

initially amplified 500x for the left-right channel and 2000x for the up-down channel and offset was adjusted to bring the signal close to zero. The signal was then passed to the other two channels on the amplifier and amplified a further 10x each for totals of 5000x and 20000x for the horizontal strain and vertical strain measurement channels respectively. Amplified voltages were recorded at 1000Hz using a NI USB-6009 board (National Instruments Corp., Austin, TX) and custom written Matlab R2013b (The MathWorks Inc., Natick, MA) scripts that utilized the Data Acquisition Toolbox. A third event channel was also recorded to synchronize the strain recordings with stimulus onset, which was signaled with an electrical pulse. All traces were saved in .csv format for further analysis in R.

**Visual stimulus:** Two types of patterns were used to create visual motion on the 0.5x0.5 meter projection surface at a resolution of 1050x1050 pixels. Stimuli were either black-and-white linear gratings with four sets of either vertical or horizontal bars (spatial frequency: 0.043 cycles per degree) or random dotfields with 250 black dots (40 pixel or 3.4 degree diameter) on a white background. Dots were initially randomly positioned and had infinite lifetimes, but would regenerate at the origin of motion (side opposite the direction of motion) if they moved off the screen. Both patterns were moved at the same speeds: 0, 2, 12, 48, 72, 84, 120 degrees per second and in the same directions: left, right, up, and down, as defined by a feeding hummingbird's point of view. Stimuli were generated and controlled using custom scripts in Matlab and utilizing Psychtoolbox-3 (Brainard, D. H., 1997; Kleiner et al., 2007; Pelli, 1997).

**Experiment procedure:** Hummingbirds were initially trained to the experimental chamber by allowing them to acclimate with a known feeder. A non-moving pattern was projected from the start of training, with four birds trained with a grating, and four with a dotfield. After their behavior settled to a normal mix of perching and flying, the known feeder was removed and the

bird was expected to begin feeding from the experimental feeder. Initially a red tube was attached to the front of the experimental feeder to visually guide the bird, but this was removed after the bird consumed sugar solution. Birds were then trained to feed on a 15 minute schedule by capping the feeder so the bird did not have access to food, then opening the feeder every 15 minutes and giving the bird up to 2 minutes to feed. Once a bird reliably followed the schedule for several hours, it was considered trained. No birds were excluded from experiments on the basis of failed training.

Two experiments were conducted on two separate days within a four day span posttraining. The first experiment was to move the background pattern in four different directions (left, right, up, and down) at 12 degrees per second. Birds were tested with the pattern they received during training. Each direction was tested twice in random order, which also included two non-moving trials. Birds were kept to a 15 minute schedule, with discretionary changes to 20 minute schedules if birds were consistently skipping trials at 15 minute intervals. At the end of each trial, the feeder was refilled from a syringe with 0.1 milliliter graduations and trials where birds fed less than 0.1 milliliters were added to a repeat list. The trials on the repeat list were conducted again at the end of the initial set of 10 stimulus treatments. Each trial began with starting the video and strain recordings, then the stimulus, and finally opening the feeder. Trials were concluded in the reverse order.

The second experiment used the same trial-to-trial procedure but with different stimuli. In this experiment all birds received a mix of the two patterns with each pattern moving to the left at seven different speeds: 0, 2, 12, 48, 72, 84, 120 degrees per second. Each speed and pattern combination was used once. Again, trials where the bird did not drink at least 0.1 milliliters were repeated after the random trial sequence had been completed.

**Analysis:** Bird presence at the feeder was determined by digitizing the video recordings. The timestamps of the bird arriving and leaving were matched to the recorded traces from the horizontal and vertical strain amplifier channels (Fig 14B-C). Portions where the bird was present and feeding were conservatively cropped by 0.25 seconds on either end to eliminate signal spikes caused by docking and undocking with the feeder. The feeding portions were analyzed relative to a minimum of 3 seconds of recording before the first feed and after the last. Each trace was detrended by subtracting a linear fit to the pre- and post-feed data. Trace analysis and statistical analysis was performed in R version 3.1.3 (R Core Team, 2015) using linear mixed models (Pinheiro et al., 2016) and post-hoc testing using Tukey tests in the multcomp package (Hothorn et al., 2008). Fixed effects included pattern, stimulus speed, and stimulus direction. Individual hummingbirds, and repeats of trials within individual were treated as random effects, with feed within a trial also included where appropriate. Spectral analysis was performed with signal (signal developers, 2013). Figures were generated with ggplot2 (Wickham, 2009) and edited in Adobe Illustrator CS4 (Adobe Systems Inc., San Jose, CA).

**Calibration:** Strain signals were calibrated by hanging 2, 5, and 10 gram weights on the feeder. The feeder could be reoriented to allow hanging weight orientations in the down, left, or right directions. Calibration points were analyzed and fit with a line through the origin. The mean of the line slopes for horizontal and vertical axis was used to relate voltage of the respective channel to mass units.

#### Results

#### Hummingbirds physically interact with the feeder during feeding

Hummingbirds physically connect and interact with the feeder when they are docked. For this section we only present analysis of experimental trials without background stimulus motion. The physical interaction with the feeder is evident through several metrics. During feeding, the amplitude of the strain signal often increases relative to pre-feeding amplitude (v min = 1.141, v median = 1.783, v max = 19.280; h min = 0.775, h median = 1.211, h max = 3.089). The amplitude change is primarily in the vertical axis (Fig. 14B-C for representative raw traces, Fig. 15A for analysis) which exhibits an average change about 2.4 times (v mean = 3.000; h mean = 1.236) greater than the change in horizontal axis amplitude ( $F_{1,100} = 44.428$ , *P* < 0.0001).

The increase in signal during docked feeding is related to several behaviorally relevant frequencies (Fig. 14D-E for representative spectrograms, Fig. 15B for analysis). Anna's hummingbirds extend their tongue to lick nectar at about 10-15Hz (Ewald and Williams, 1982) and spectral analysis of strain recordings exhibit increased power in this frequency range when the bird is docked (v min = 0.865, v mean = 4.991; h min = 0.612, h.mean = 1.956). Hovering wingbeat frequency around 35 to 45Hz (Altshuler et al., 2012; Clark and Dudley, 2010) is also transmitted to the feeder (v min = 1.062, v mean = 4.683; h min = 0.785, h.mean = 1.768) and is always apparent in the vertical strain measurements. For comparison, the power of the signal at 60Hz, electrical background noise, is relatively unchanged (v min = 0.775, v mean = 1.096; h min = 0.454, h mean = 1.047). The licking and wingbeat frequencies are transmitted significantly more in the vertical axis than the horizontal axis (10-15Hz:  $F_{1,100} = 17.725$ , *P* < 0.0001; 35-45Hz:  $F_{1,100} = 75.862$ , *P* < 0.0001) but not the electrical noise (58-63Hz:  $F_{1,100} = 2.616$ , *P* = 0.109).

In addition to the high frequency interactions, hummingbirds also push against the feeder. The measurement to illustrate this pushing interaction is a difference between the feeding mean, and the pre-feeding signal mean. Individual hummingbirds vary in how much they push against the feeder during feeding, with no background visual motion, and also how much variance they exhibit from feed to feed (Fig 15C). However, this is not systematic and there is no effect of pattern ( $F_{1,52} = 1.432$ , P = 0.237) or day ( $F_{1,19} = 0.791$ , P = 0.385) on horizontal push. Similarly, vertical push is not affected by background pattern ( $F_{1,52} = 0.788$ , P = 0.389) or day ( $F_{1,19} = 1.982$ , P = 0.175).

#### Hummingbirds stabilize directional visual motion during feeding

Feeding hummingbirds push against the feeder in the direction of visual motion but remain docked. We analyzed horizontal push (Fig. 16A) during trials with moving backgrounds using a linear mixed effects model and found an interaction between pattern and stimulus motion direction ( $F_{4,125} = 3.279$ , P = 0.0136). As a result, we split the analysis by stimulus pattern and found that direction of stimulus motion has an effect with both gratings ( $F_{4,68} = 13.120$ , P <0.0001) and dotfields ( $F_{4,57} = 14.305$ , P < 0.0001). Post-hoc Tukey tests were used to compare the response to the different direction treatments. Dotfields moving right and left had a strong effect on how much a hummingbird pushed right and left (right: estimate: -6.894, P < 0.001; left: estimate: 5.184, P = 0.015). In contrast, gratings only elicited significant pushing to the right (estimate: -7.314, P < 0.001), but the lack of a significant leftward response (estimate: 0.714, P =0.981) can be explained by the leftward push in no-motion trials with the gratings. The horizontal response to up and downward moving gratings further indicated the leftward preference in the treatment without visual motion. While the horizontal response to gratings moving in both vertical directions was close to zero (as we predicted for no motion and vertical motion), down was significantly different from no motion (estimate: -4.228, P = 0.00495) and up was not (estimate: -3.0491, P = 0.111). With dotfields, up and down matched predictions as they were not different from the no motion treatment (up estimate: 2.577, P = 0.442; down estimate: 0.428, P = 0.998).

Vertical push (Fig. 16B) also exhibited an interaction between pattern and visual motion direction ( $F_{4,125} = 2.620$ , P = 0.0381) so the analysis was again split by pattern. Dotfields again had a stronger effect on the hummingbirds' pushing response and visual motion direction had an effect ( $F_{4,57} = 9.377$ , P < 0.0001). Interestingly, hummingbirds only stabilized downward motion (estimate: -17.528, P < 0.001), there was no vertical pushing response to visual motion moving up, left, or right. With the grating patterns, visual motion had no effect on vertical pushing ( $F_{4,68}$ = 1.633, P = 0.176).

#### Visual motion stabilizing response in feeding hummingbirds is speed tuned

We also tested a single motion direction at a range of speeds to determine if the hummingbird pushing response was speed tuned. Speed tuning was again analyzed with a linear mixed effects model, this time using speed as the fixed effect instead of direction. All motion was to the left in this experiment. Analysis of the horizontal push yielded a significant effect of speed ( $F_{6,166} = 10.118$ , P < 0.0001) but not pattern ( $F_{1,166} = 2.516$ , P = 0.115) and no interaction between speed and pattern ( $F_{6,166} = 1.276$ , P = 0.271). We removed the interaction term and repeated the analysis, then conducted a Tukey test to determine differences between speeds (Fig. 17). Speeds of 2 and 120 degrees per second were not significantly different from no motion (estimate = -0.134, P = 1.0; estimate = 2.257, P = 0.247; respectively). Leftward motion at speeds of 12-84 degrees per second were significantly different from no motion (estimates:

3.782-5.623, P < 0.00475). Over the range tested, the response appeared to peak between 12-48 degrees per second.

In the vertical direction, where no response was expected, the interaction term was not significant ( $F_{6,166} = 1.818$ , P = 0.0983) but visual motion speed did have a significant effect ( $F_{6,166} = 2.349$ , P = 0.0333). We removed the interaction term from the model, even though this was not necessarily warranted, to enable post-hoc testing to probe the significant speed fixed effect. Tukey contrasts yielded no significant speed contrasts (estimates: -3.611 to 5.277, all  $P \ge 0.0693$ ). This suggested that a single contrast between 2 and 120 degrees per second (estimate: 5.276, P = 0.0693) was driving the speed significance in the model, but that there were no systematic differences indicative of a consistent vertical response.

#### Discussion

We investigated how a hummingbird pushes against a feeder once it has docked by measuring horizontal and vertical forces exerted on an artificial feeder by feeding hummingbirds. We first measured hummingbird interactions with a feeder without manipulating the visual background and found that hummingbirds push against the feeder when they open their bill to drink, beat their wings, and when they shift their body or head position. Hummingbirds feeding in front of moving visual patterns also push against the feeder in the direction of the background motion, with the exception of upward motion. The stabilization response is speed tuned and quickly saturates. Our results indicate that bill contact with the feeder does not eliminate the feeding hummingbird's attempts to stabilize visual motion, suggesting that fusion of vision and touch does not strongly modify position control during docked hovering. Behavioral reactions to the direction of translational and rotational optic flow are common in animals. Humans exhibit strong reactions to optic flow as infants, even falling forward or backward because of visual motion (Lee and Aronson, 1974). Over time, and with practice, other sensors are incorporated and control becomes more refined (Lee, 1980). Adults exhibit differences in how they weight tactile and visual signals, and also their sensitivity to conflicts between the two senses (Jeka et al., 2000). It is surprising that hummingbirds push against the feeder because we predicted that docking would provide a second source of information about positional stability, much as touch helps to stabilize human posture sway. Designing a new experiment to compare the gain and phase of stabilization responses during free and docked hovering would potentially answer whether touch can attenuate the visual stabilization response. The absence of an upward stabilization response suggests that docking does modify hummingbird hovering control.

Docked hummingbirds do not push upwards to stabilize visual motion. The absence of an upward stabilization response is more likely to be the result of a behavioral trade-off than a physical limitation or visual processing asymmetry. Hummingbirds can lift at least 150% of their body weight (Altshuler et al., 2010; Chai et al., 1997), and pushed in other directions, so pushing upward on the feeder was physically possible. Zebra finches exhibit an enhanced optokinetic response, where the head moves to stabilize visual motion on the retina, in response to temporonasal (forward) visual motion over the opposite direction (Eckmeier and Bischof, 2008). The behavioral result matches temporonasal preferences of directionally sensitive visual motion processing neurons in the avian brain (Winterson and Brauth, 1985). However, freely hovering hummingbirds adjust body position to stabilize directional motion in all directions, including upward motion and no preference was detected (Goller and Altshuler, 2014). During feeding, the

hummingbird must open and close its bill to lick and collect nectar captured by the tongue (Rico-Guevara and Rubega, 2011), so pushing upward may prevent the feeding motion while downward or lateral pushes do not. Docked, hovering hummingbirds may experience a trade-off between stabilizing upward motion and feeding. If motion stabilization can be eliminated in the upward direction, why push against the feeder in other directions?

We measured transmission of licking and wingbeat motion primarily in the vertical axis of the feeder. During feeding, hummingbirds open and close the tips of their bills (Ewald and Williams, 1982; Rico-Guevara and Rubega, 2011). Measuring a signal at licking frequency of 10-15Hz suggests that the feeding hummingbird is bumping its bill into the feeder with each lick. Feeder shape possibly contributes to the bill-feeder interaction, but studies of feeding hummingbirds have not previously described feeder or flower shape interfering with bill motion during feeding. The vertical wingbeat frequency signal most likely comes from the vertical body oscillation during flapping flight. Bird head and eye stabilization reflexes increase their gain during fictive flight in comparison to standing (Maurice, 2006; McArthur and Dickman, 2011), and hovering birds are excellent examples of head stability (Frost, 2009). We suggest that the feeder sensor is measuring head stabilization error during hovering. Further experiments are necessary to determine how head stabilization may change with a non-rigid feeder or more complex hummingbird-flower interactions.

The horizontal stabilization response was found to be tuned to motion speed, but not specialized for slow speeds. An important visual motion processing center in birds is the nucleus lentiformes mesencephali, and it is enlarged in hummingbirds. It was predicted that this hypertrophy could be related to a need to process slow visual motion for hovering control (Iwaniuk and Wylie, 2007). In pigeons, neurons in the same nucleus have direction and speed

preferences. Neurons are classified as either 'slow' or 'fast', with slow neurons preferring speeds < 6 degrees per second (Winterson and Brauth, 1985; Wylie and Crowder, 2000). We find no evidence that hummingbirds are sensitive to motion slower than 6 degrees per second. A previous study with Ruby-throated hummingbirds did not test slow speeds, but the hummingbirds were shown to track square-wave gratings (spatial frequency 0.04 cycles per degree) moving at 62-134 degrees per second with head and body rotations (Ros and Biewener, 2016). Our results with docked hummingbirds suggest that the docked response is present over a similar range, though we found evidence that it was declining at 120 degrees per second. We also found no difference between square-wave grating and dotfield response curves for speed tuning, suggesting that the response we measured was not specific to the spatial frequency of our grating pattern. We did not test different spatial frequencies of gratings, however, and the overlap could be a coincidence.

In the wild, where hummingbirds are feeding from flowers on flexible stems, the hummingbird may be able to move and continue feeding by steering the flower. How docked hovering is controlled in a more dynamic system like the more natural flower-hummingbird interaction remains to be examined. However, this experiment is the first time that the hummingbird's interaction with the physical feeder structure has been investigated. Our results suggest that hummingbirds push against the feeder in several ways during feeding, even though the bird appears stationary as it hovers. We have shown that hummingbirds have complex interactions with the feeder during docked hovering, which has not been addressed in studies that use feeders to elicit hovering and other flight behaviors and should be considered when discussing the aerodynamics of these behaviors. Approaching and docking with a flower is

certainly a precise behavior, but our results suggest that hovering at the flower afterward may not be as precise as it looks.

# Figures



Figure 14. Experimental rig was designed to measure how hovering hummingbirds interact with the feeder. The flight chamber (A) is a 0.5m cube with a back-projection screen on one wall and a feeder centered in front of it. Horizontal and vertical strain, as well as video, are recorded and a computer also controls the motion of a background visual stimulus. Strain recordings measure pushing against the feeder in body weight units (assuming an average 4g Anna's hummingbird) for both left-right (horizontal axis, B) and up-down (vertical axis, C) pushes. Blue highlights sections where the bird is not present, while red indicates sections where the bird is docked. The two traces shown are examples where the background is stationary. Spectral analysis of the horizontal (D) and vertical (E) axis traces (B,C respectively) show the appearance of the wingbeat (~40Hz) and more faint licking (10-15Hz) frequency bands when the bird is docked.



**Figure 15. Docked hummingbirds interact with the feeder.** Data shown here are from trials without stimulus motion. Strain measurements show that hummingbirds push against the feeder in multiple ways. The amplitude of the recording (A) increases when the hummingbird docks, especially in the vertical axis. Both axes exhibit signatures of two behaviorally relevant frequencies (B): licking frequency (10-15Hz) and wingbeat frequency (35-45Hz). In comparison to the electrical noise that is not bird-related, these frequencies appear in the strain recordings after the bird docks and suggest that the bird's bill transmits body motions. Finally the bird also adjusts its body or head position to push against the feeder (C). These pushes are highly variable both within and between individuals, but there is no systematic trend across days or between background visual patterns. The faded symbols show the raw data, and the full-color squares show daily means.



#### Figure 16. Hummingbirds attempt to stabilize visual motion during docked feeding,

**especially in the horizontal axis.** Horizontal (A) and vertical (B) push was measured each time a hummingbird docked, and each bird was presented with right-, left-, up-, and down-moving patterns, as well as stationary ones, during feeding. Black arrows indicate the expected push direction if the bird attempts to stabilize directional motion. In both axes the gratings (red) had less of an effect than the dotfields (blue). In most cases hummingbirds docked multiple times within a single stimulus trial (multiple feeds within a trial) and this is indicated by the increasing diameter of the raw data symbols. Overall means of all feeds in a stimulus and pattern group are shown by the plus symbols. Hummingbirds push against the feeder in an attempt to stabilize right, left, and down motion, but there is no evidence that they attempt stabilize up motion.



**Figure 17. Hummingbird attempts to stabilize leftward visual motion exhibit speed tuning.** Horizontal push was measured for docked hummingbirds feeding in front of gratings and dotfields moving to the bird's left at different speeds from 0 to 120 degrees per second. Raw data are shown as circles, with overall means as the plus symbols. Means are joined by colored lines to visualize the tuning curve. The individual tuning curves for each of the eight individual birds are shown in gray. These individual lines combine the responses for the two patterns. Overall, birds exhibit a response plateau by 12 degrees per second that then declines for motion faster than 84 degrees per second. No response was measured for patterns moving at 2 degrees per second.

# CONCLUSION

Collecting nectar, and hovering at the flower to do so, is central to hummingbird behavior, ecology, and evolution (Altshuler and Dudley, 2002). Hummingbird ability to sustain hovering flight is unique among birds, and hummingbird morphology and physiology are specialized for the energetic and aerodynamic demands of hovering. It follows therefore, that sensory control of flight in hummingbirds would similarly be tuned to facilitate hovering at flowers, and specialization of the hummingbird brain suggests that visual motion perception is especially important for this behavior (Iwaniuk and Wylie, 2007). This dissertation shows that hovering hummingbirds are sensitive to visual motion, and suggests that hovering control is not tuned for maintaining bird-flower interaction but rather for minimizing perceived motion in space. We anticipated that hovering stability at the flower would be difficult to alter but found that hummingbirds continue to respond to moving backgrounds even when birds are docked with a feeder. It is remarkable that hummingbirds exhibit such sensitivity to background motions during a flight behavior that allows hummingbirds to feed from flowers.

The studies comprising this dissertation are on a short, but recently expanding, list of behavioral studies investigating visual control of avian flight. The strength of hummingbird behavioral responses during free hovering in a virtual reality arena has helped to draw attention to avian models and to bridge avian visual neuroscience with flight (Wylie et al., 2015). In the avian brain large-field visual motion, or optic flow, is processed by a pathway named the accessory optic system. Two major, complementary, nuclei in this pathway are called the nucleus of the basal optic root (nBOR) and the lentiformes mesencephali (LM). These nuclei provide visual motion processing underlying avian eye, head, and body reflexes that serve to minimize perceived motion on the retina and thereby facilitate visual acuity and body stability. The LM is enlarged in hummingbirds while the nBOR is not, suggesting a functional importance of the LM, and optic flow, for controlling body position during hovering (Iwaniuk and Wylie, 2007). Neurons in the LM prefer temporonasal (back-to-front) visual motion and reflexive motions to stabilize retinal images are accordingly enhanced for visual motion in the forward direction (Eckmeier and Bischof, 2008; Gioanni, 1988a). Other animals exhibit similar asymmetry (e.g. Collewijn, 1980; Masseck et al., 2008). This dissertation shows that hovering hummingbirds are highly sensitive to optic flow, implicating the LM in hovering control, as had been previously predicted (Iwaniuk and Wylie, 2007).

Hummingbirds did not exhibit behavioral asymmetry in flight responses for the visual motion directions tested, as predicted by LM neuron direction preferences. One factor may have been binocular viewing of the stimuli, which was shown to eliminate asymmetry of responses to lateral motion in zebra finches (Eckmeier and Bischof, 2008). However, for forward or temporonasal motion, which is preferred by LM neurons, binocularity increased responses in humans (Berthoz et al., 1975; Lestienne et al., 1977). Making finer measurements of stabilizing behavior, including motions of the eyes, head, and body, in hovering hummingbirds would be important to determine whether behavioral asymmetries exist. Alternatively, our results could suggest that the hummingbird brain is more specialized for motion perception than previously thought, or that motion processing is different when the animal is in flight in comparison to stationary on the laboratory bench.

Birds exhibit differences in body and head stabilization reflexes depending on their behavioral state. In addition to passive damping in the neck (Pete et al., 2015), active compensation helps to keep the head and eyes stable during flight, a critical prerequisite for using vision to control flight where the body oscillates with each wingbeat (Gioanni, 1988a,

1988b; Gioanni and Vidal, 2012; Wylie and Frost, 1996). During flight, wing and tail reflexes help to correct sudden changes in body position in concert with head and eye stabilization reflexes. These control reflexes are significantly damped, or absent, during perching and standing, compared to a fictive flight preparation (Bilo and Bilo, 1978; McArthur and Dickman, 2011). Behavioral state is important for numerous reflexes, but we know little about how behavioral state affects sensory processing in the brain.

Stabilization reflexes are enhanced during active behavior suggesting that visual motion detection pathways underlying optomotor reflexes may be similarly enhanced. Other types of visual motion detection or guidance, such as circuits governing interactions with objects in the environment, may not change with behavioral state and may be universally relevant. Visual expansion is an important signal for controlling landing (Lee et al., 1993), diving (Lee and Reddish, 1981), and even hummingbird docking (Lee et al., 1991), but is also important to detect an approaching predator. Descriptive studies used bird behavior as examples to support predictions about important visual parameters, such as relative rate of visual expansion, and the predicted parameters were later found to be encoded by neurons in the tectofugal pathway of birds (Sun and Frost, 1998). Recent flight studies with birds have further strengthened the early descriptive work (Bhagavatula et al., 2009; Vo et al., 2016). There is a strong background of knowledge on avian visual motion processing that provides hypotheses for behavior experiments, but can also benefit from identification of important visual parameters through studies of flight control in birds.

In addition to complementing research on avian motion processing, the studies of hummingbird flight in this dissertation contribute to an increasingly interesting comparison of visual flight control mechanisms in insects and birds. Hummingbird sensitivity to motion and

persistent stabilization of motion once docked are surprising, but the general strategy of stabilizing visual motion during hovering was also shown in insects. Hovering insects presented with moving backgrounds control their position in space by moving their bodies to stabilize visual motion (Farina et al., 1994; Kelber and Zeil, 1990, 1997; Kern and Varjú, 1998). Like the hummingbirds, these insects stabilize visual motion during a hovering behavior involving a target. In contrast to docked hovering in hummingbirds however, hawkmoths and guard bees are able to move relative to their targets because they are not rigidly docked. Hovering moths feed with a long, flexible proboscis that gives them the freedom to track flower and visual background motion without losing contact with the nectar (Farina et al., 1994; Kern and Varjú, 1998; Sprayberry and Daniel, 2007). Guard bees holding positions near their nest entrance are not physically connected to their target and are free to drift (Kelber and Zeil, 1990, 1997; Zeil and Wittmann, 1989), much like a hummingbird during a look-up. Once docked however, hummingbirds are anchored by their rigid bill yet still attempt to stabilize visual motion (Chapter 3). The docked attempts to stabilize motion suggest that there may be a different interaction between feeding hummingbirds and flowers than the flower tracking shown in hawkmoths. Hummingbirds could "track" flower oscillations but could also resist flower motions, holding the flowers in a moving environment or even steering them. Most laboratory studies with hummingbirds have used rigid syringes or plastic feeders in the place of natural flowers and it would be critical to also test flexible feeders for future hovering studies.

While hovering hummingbirds and insects respond to visual motion in similar ways during hovering, we cannot generally assume that visual guidance strategies converge between insects and birds. Numerous recent studies have focused on testing stimuli that are important for insect flight control on flying birds. For example bees and bumblebees use optic flow to control

velocity and navigate through a tunnel under both laboratory and naturalistic conditions (Baird et al., 2006; Baird and Dacke, 2012; Srinivasan et al., 1991). Budgerigars were found to change flight course like the bees when traversing a tunnel (Bhagavatula et al., 2011) and also to regulate flight speed based on optic flow, although the velocity changes only occurred for increasing flight speeds. The directional bias in visual velocity control suggests that budgerigars, unlike the bees, may be limited by energetic or gait constraints (Schiffner and Srinivasan, 2015). Hummingbirds in a flight tunnel did not use the same optic flow signals to navigate, instead relying on visual expansion of large vertical features to determine lateral position (Dakin et al., 2016). The different results between bird species, insects, and recently also fish (Scholtyssek et al., 2014), illustrate the importance of studies that empirically test behavior control. In addition, it is important to interpret the laboratory studies in the context of natural environments and behaviors, and to attempt to explain how other visual information or senses may influence behavior control.

Properties of the visual system are important considerations when explaining visual control of behavior. The behavior experiments in this dissertation contributed to an interest in the morphology and resolution of the hummingbird visual system. Hummingbirds have two retinal specializations, a temporal area facing forward and a fovea projecting laterally (Lisney et al., 2015; Wood, 1917). Receptor densities suggest that hummingbirds have spatial resolution of 5 to 6 cycles per degree (Lisney et al., 2015) and these anatomical results match behavioral experiments indicating spatial acuity between 4.95 and 6.18 cycles per degree. Temporal resolution was determined to be between 70 and 80 Hz (Fellows, 2015). How these properties of the eye translate into flight control largely remains to be explored, but they help to interpret behavior experiments. During forward flight, a lack of response to vertical gratings with high

spatial frequency can be explained by fusion of the grating stripes (Dakin et al., 2016). Docked hummingbirds oscillate when feeding in front of a looming spiral pattern, a behavior characterized by backward drifts away from the feeder followed by forward flight to recover docked position. Despite the impact of visual motion on hovering, hummingbirds are able to reliably approach and dock with the feeder, perhaps implicating different parts of the retina and different motion sensitivity for the hovering and docking components of hummingbird feeding.

This dissertation focuses on a single flight task, maintaining position during hovering, which is key to hummingbird behavior. Although the experiments are motivated by questions about hovering control in natural environments, the experiments were nevertheless conducted with unnatural, black-and-white stimulus patterns, primarily square-wave gratings and spirals. Initially, in the absence of previous work on visual flight control in hummingbirds, we attempted to change hummingbird hovering stability using psychophysics stimuli that had previously been used with other animals (e.g. Bhagavatula et al., 2011; Braitenberg and Ferretti, 1966; Kelber and Zeil, 1990; Kern and Varjú, 1998; Martinoya and Delius, 1990). We found that responses to visual motion were strong and designed further experiments to investigate the extent to which hummingbirds relied on visual motion to control hovering. In the process, many questions about how the flight response matched stimulus properties, and how the stimulus patterns relate to natural environments have gone unanswered. Hummingbird hovering is a good candidate behavior for studies testing limits of hummingbird visual perception. Stimulus parameters such as spatial and temporal frequency, contrast, velocity, direction, and color could be manipulated to determine limits of the visual system and motion processing pathways. Hummingbirds may use elementary motion detector-like visual processing to generate optomotor responses, which would then be tuned to temporal frequency not motion velocity. Behavioral limits could also be

tested, such as how quickly the hummingbird responds to changes in direction, and whether there are any preferred directions such as those matching the orientation of the semi-circular canals (Wylie et al., 1998).

Over the course of the experiments in this dissertation, hummingbird behavior patterns unrelated to the visual stimulus treatment became apparent. Hummingbirds exhibited a preference for the side of experimental chamber away from the chamber door or experimenter. This preference is apparent in the non-zero horizontal angle of response drifts and lateral movements of hummingbirds during treatments without stimulus motion in all three chapters. In addition, hummingbirds were always able to precisely dock with the feeder, even in the presence of immersive visual motion stimuli. The accuracy of body positioning and control during approach flight and feeder docking suggests that placing the bill into a small, clear plastic target involves completely different visual control mechanisms than position control during hovering, and that docking control is insensitive to background visual motion. Understanding the way visual motion is used when docking, in contrast to motion sensitivity once docked, may provide important insight into the simultaneous processing of visual motion in the accessory optic system and tectofugal pathways (Frost, 2010).

Hummingbirds are able to dock and feed from flowers in complex and dynamic natural settings, including flowers moving in the wind. Studies of avian flight biomechanics have recently emphasized maneuvering performance and kinematic control of maneuvers. Hummingbirds have been a popular study organism for such studies because they can be trained to feed in wind tunnels or to follow moving feeders to elicit maneuvers such as backward flight (Sapir and Dudley, 2012), turning in place (Altshuler et al., 2012), arcing flight (Read et al., 2016), or flying in turbulence (Ravi et al., 2015). These studies measure wing motion under the

assumption that the feeding hummingbirds are generating the forces necessary to perform the intended maneuver. Experiments in this dissertation suggest that there are complex interactions at play during docked flight, including physical interaction between the docked hummingbird and the feeder, and the influence of these interactions on the motion of the bird should be addressed in studies that seek to relate wing motions to body motion. Experimental measurements of hovering show that hummingbirds are not as stable as they may appear, and that rigid feeders enforce and support the apparent precision and stability of hovering hummingbirds.

Birds offer an exciting new perspective on visual control of flight that complements behavior work in insect systems and advances in avian neurobiology. Hummingbirds have a particularly tractable flight behavior for laboratory experiments, and this dissertation shows that they are responsive to visual motion. Their sensitivity to visual background manipulation provides ample opportunity to test the parameters and limitations of vision on behavior. Birds are important models for studies seeking to integrate vision, behavior, and neural control. Advances in technology like eye tracking (Yorzinski et al., 2015; Yorzinski and Platt, 2013) and neurophysiological recording in freely behaving animals will be critical to facilitate this research. Hummingbirds may be too small for early implementation of these technologies, but this dissertation shows that they are sensitive to visual motion and translate that sensitivity directly into consistent changes in hovering behavior. They are excellent candidates for further studies bridging bird brains, vision, and behavior.

### REFERENCES

- Altshuler, D. L., and Dudley, R. (2002). The ecological and evolutionary interface of hummingbird flight physiology. *J. Exp. Biol.* 205, 2325–2336.
- Altshuler, D. L., Dudley, R., Heredia, S. M., and McGuire, J. A. (2010). Allometry of hummingbird lifting performance. *J. Exp. Biol.* 213, 725–734.
- Altshuler, D. L., Quicazan-Rubio, E. M., Segre, P. S., and Middleton, K. M. (2012). Wingbeat kinematics and motor control of yaw turns in Anna's hummingbirds (*Calypte anna*). J. *Exp. Biol.* 215, 4070–4084.
- Baird, E., Boeddeker, N., Ibbotson, M. R., and Srinivasan, M. V. (2013). A universal strategy for visually guided landing. *Proceedings of the National Academy of Sciences* 110, 18686– 18691. doi:10.1073/pnas.1314311110.
- Baird, E., and Dacke, M. (2012). Visual flight control in naturalistic and artificial environments. *J. Comp. Phys. A* 198, 869–876.
- Baird, E., Kreiss, E., Wcislo, W., Warrant, E., and Dacke, M. (2011). Nocturnal insects use optic flow for flight control. *Biol. Lett.* 7, 499–501.
- Baird, E., Srinivasan, M. V., Zhang, S., Lamont, R., and Cowling, A. (2006). "Visual control of flight speed and height in the honeybee," in *From Animals to Animats 9*, 40–51.
- Barnett, P. D., Nordström, K., and O'Carroll, D. C. (2010). Motion adaptation and the velocity coding of natural scenes. *Current Biology* 20, 994–999.
- Bender, J. A., and Dickinson, M. H. (2006a). A comparison of visual and haltere-mediated feedback in the control of body saccades in *Drosophila melanogaster*. J. Exp. Biol. 209, 4597–4606. doi:10.1242/jeb.02583.
- Bender, J. A., and Dickinson, M. H. (2006b). Visual stimulation of saccades in magnetically tethered *Drosophila*. J. Exp. Biol. 209, 3170–3182. doi:10.1242/jeb.02369.
- Berthoz, A., Pavard, B., and Young, L. R. (1975). Perception of linear horizontal self-motion induced by peripheral vision (linearvection) basic characteristics and visual-vestibular interactions. *Experimental Brain Research* 23, 471–489.
- Bhagavatula, P. S., Claudianos, C., Ibbotson, M. R., and Srinivasan, M. V. (2011). Optic flow cues guide flight in birds. *Current Biology* 21, 1794–1799.
- Bhagavatula, P. S., Claudianos, C., Ibbotson, M., and Srinivasan, M. V. (2009). Edge detection in landing budgerigars (*Melopsittacus undulatus*). *PLoS ONE* 4, e7301. doi:10.1371/journal.pone.0007301.

- Bilo, D., and Bilo, A. (1978). Wind stimuli control vestibular and optokinetic reflexes in the pigeon. *Naturwissenschaften* 65, 161–162.
- Borst, A., and Haag, J. (2002). Neural networks in the cockpit of the fly. J. Comp. Phys. A 188, 419–437.
- Borst, A., Haag, J., and Reiff, D. F. (2010). Fly motion vision. *Annual Review of Neuroscience* 33, 49–70.
- Brainard, D. H. (1997). The Psychophysics toolbox. Spatial Vision 10, 443-446.
- Braitenberg, V., and Ferretti, C. T. (1966). Landing reaction of *Musca domestica* induced by visual stimuli. *Naturwissenschaften* 53, 155–155.
- van Breugel, F., and Dickinson, M. H. (2012). The visual control of landing and obstacle avoidance in the fruit fly *Drosophila melanogaster*. J. Exp. Biol. 215, 1783–1798.
- Chai, P., Chen, J. S., and Dudley, R. (1997). Transient hovering performance of hummingbirds under conditions of maximal loading. *J. Exp. Biol.* 200, 921–929.
- Chai, P., and Dudley, R. (1995). Limits to vertebrate locomotor energetics suggested by hummingbirds hovering in heliox. *Nature* 377, 722–725.
- Chen, D. M., and Goldsmith, T. H. (1986). Four spectral classes of cone in the retinas of birds. J. *Comp. Phys. A* 159, 473–479.
- Chow, D. M., and Frye, M. A. (2008). Context-dependent olfactory enhancement of optomotor flight control in *Drosophila*. J. Exp. Biol. 211, 2478–2485. doi:10.1242/jeb.018879.
- Clark, C. J., and Dudley, R. (2010). Hovering and forward flight energetics in Anna's and Allen's hummingbirds. *PBZ* 83, 654–662.
- Coggshall, J. C. (1972). The landing response and visual processing in the milkweed bug, *Oncopeltus fasciatus. J. Exp. Biol.* 57, 401–413.
- Collett, T. S. (1980). Some operating rules for the optomotor system of a hoverfly during voluntary flight. *J. Comp. Phys. A* 138, 271–282.
- Collett, T. S., and Land, M. F. (1975). Visual control of flight behaviour in the hoverfly, *Syritta pipiens* L. *J. Comp. Phys.* 99, 1–66.
- Collewijn, H. (1980). Sensory control of optokinetic nystagmus in the rabbit. *Trends in Neurosciences* 3, 277–280.
- Dakin, R., Fellows, T. K., and Altshuler, D. L. (2016). Visual guidance of forward flight in hummingbirds reveals control based on image features instead of pattern velocity. *PNAS* 113, 8849–8854. doi:10.1073/pnas.1603221113.
- David, C. T. (1979). Height control by free-flying Drosophila. Physiol. Ent. 4, 209–216.

- De Talens, F. P., and Ferretti, C. T. (1970). Landing reacation of *Musca domestica*: dependence on dimensions and position of the stimulus. *J. Exp. Biol.* 52, 233–256.
- Dickinson, M. H., Farley, C. T., Full, R. J., Koehl, M. A. R., Kram, R., and Lehman, S. (2000). How animals move: an integrative view. *Science* 288, 100–106.
- Donovan, E. R., Keeney, B. K., Kung, E., Makan, S., Wild, J. M., and Altshuler, D. L. (2013). Muscle activation patterns and motor anatomy of Anna's hummingbirds *Calypte anna* and Zebra finches *Taeniopygia guttata*. *PBZ* 86.
- Dubois, M. F. W., and Collewijn, H. (1979). The optokinetic reactions of the rabbit: relation to the visual streak. *Vision Res.* 19, 9–17.
- Eckmeier, D., and Bischof, H.-J. (2008). The optokinetic response in wild type and white zebra finches. J. Comp. Phys. A 194, 871–878. doi:10.1007/s00359-008-0358-7.
- Eckmeier, D., Geurten, B. R. H., Kress, D., Mertes, M., Kern, R., Egelhaaf, M., et al. (2008). Gaze strategy in the free flying zebra finch (*Taeniopygia guttata*). *PLoS ONE* 3, e3956.
- Egelhaaf, M., Kern, R., and Lindemann, J. P. (2014). Motion as a source of environmental information: a fresh view on biological motion computation by insect brains. *Frontiers in Neural Circuits* 8. doi:10.3389/fncir.2014.00127.
- Ewald, P. W., and Williams, W. A. (1982). Function of the bill and tongue in nectar uptake by hummingbirds. *The Auk* 99, 573–576.
- Farina, W. M., Varju, D., and Zhou, Y. (1994). The regulation of distance to dummy flowers during hovering flight in the hawk moth *Macroglossum stellatarum*. J. Comp. Phys. A 174, 239–247.
- Fellows, T. K. (2015). Visual resolution of Anna's hummingbirds (*Calypte anna*) in space and time. M.Sc. Thesis. University of British Columbia, Vancouver.
- Fetsch, C. R., Turner, A. H., DeAngelis, G. C., and Angelaki, D. E. (2009). Dynamic reweighting of visual and vestibular cues during self-motion perception. *Journal of Neuroscience* 29, 15601–15612.
- Finkelstein, A., Derdikman, D., Rubin, A., Foerster, J. N., Las, L., and Ulanovsky, N. (2015). Three-dimensional head-direction coding in the bat brain. *Nature* 517, 159–164.
- Frost, B. J. (2009). Bird head stabilization. *Current Biology* 19, R315–R316. doi:10.1016/j.cub.2009.02.002.
- Frost, B. J. (2010). A taxonomy of different forms of visual motion detection and their underlying neural mechanisms. *Brain Behav. Evol.* 75, 218–235. doi:10.1159/000314284.
- Frost, B. J., Wylie, D. R. W., and Wang, Y.-C. (1990). The processing of object and self-motion in the tectofugal and accessory optic pathways of birds. *Vision Res.* 30, 1677–1688.

- Frye, M. A. (2010). Multisensory systems integration for high-performance motor control in flies. *Current Opinion in Neurobiology* 20, 347–352. doi:10.1016/j.conb.2010.02.002.
- Frye, M. A., Tarsitano, M., and Dickinson, M. H. (2003). Odor localization requires visual feedback during free flight in *Drosophila melanogaster*. J. Exp. Biol. 206, 843–855. doi:10.1242/jeb.00175.
- Gaunt, A. S., and Gans, C. (1993). Variations in the distribution of motor end-plates in the avian pectoralis. *Journal of Morphology* 215, 65–88.
- Gibson, J. J. (1950). The perception of the visual world. Cambridge, MA: The Riverside Press.
- Gibson, J. J. (1954). The visual perception of objective motion and subjective movement. *Psychological Review* 61, 304–314.
- Gibson, J. J. (1958). Visually controlled locomotion and visual orientation in animals. *British Journal of Psychology* 49, 182–194.
- Gioanni, H. (1988a). Stabilizing gaze reflexes in the pigeon (*Columba livia*) i: horizontal and vertical eye and head reflexes. *Experimental Brain Research* 69, 567–582.
- Gioanni, H. (1988b). Stabilizing gaze reflexes in the pigeon (*Columba livia*) ii. vestibulo-ocular (VOR) and vestibulo-collic (closed-loop VCR) reflexes. *Experimental Brain Research* 69, 583–593.
- Gioanni, H., and Vidal, P.-P. (2012). Possible cues driving context-specific adaptation of optocollic reflex in pigeons (Columba livia). *Journal of Neurophysiology* 107, 704–717. doi:10.1152/jn.00684.2011.
- Goller, B. (2011). Visual control of station-holding in hummingbirds. M.Sc. Thesis. University of California, Riverside.
- Goller, B., and Altshuler, D. L. (2014). Hummingbirds control hovering flight by stabilizing visual motion. *Proceedings of the National Academy of Sciences* 111, 18375–18380.
- Greenewalt, C. H. (1962). Dimensional relationships for flying animals. *Smithsonian Miscellaneous Collections* 144, 1–46.
- Hagiwara, S., Chichibu, S., and Simpson, N. (1968). Neuromuscular mechanisms of wing beat in hummingbirds. J. Comp. Phys. A 60, 209–218.
- Holden, M., Ventura, J., and Lackner, J. R. (1994). Stabilization of posture by precision contact of the index finger. *J. Vestib. Res.* 4, 285–301.
- Hopp, E., Borst, A., and Haag, J. (2014). Subcellular mapping of dendritic activity in optic flow processing neurons. J. Comp. Phys. A 200, 359–370.

- Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal* 50, 346–363.
- Hwang, S., Agada, P., Kiemel, T., and Jeka, J. J. (2014). Dynamic reweighting of three modalities for sensor fusion. *PLoS ONE* 9, e88132.
- Iwaniuk, A. N., and Wylie, D. R. W. (2007). Neural specialization for hovering in hummingbirds: hypertrophy of the pretectal nucleus lentiformis mesencephali. J. Comp. Neurol. 500, 211–221.
- Jeka, J. J., and Lackner, J. R. (1994). Fingertip contact influences human postural control. *Exp. Brain Res.* 79, 495–502.
- Jeka, J. J., Oie, K. S., and Kiemel, T. (2000). Multisensory information for human postural control: integrating touch and vision. *Exp. Brain Res.* 134, 107–125. doi:10.1007/s002210000412.
- Junger, W., and Dahmen, H. J. (1991). Response to self-motion in waterstriders: visual discrimination between rotation and translation. J. Comp. Phys. A 169, 641–646.
- Kelber, A., and Zeil, J. (1990). A robust procedure for visual stabilisation of hovering flight position in guard bees of *Trigona (Tetragonisca) angustula* (Apidae, Meliponinae). J. Comp. Phys. A 167, 569–577.
- Kelber, A., and Zeil, J. (1997). *Tetragonisca* guard bees interpret expanding and contracting patterns as unintended displacement in space. J. Comp. Phys. A 181, 257–265.
- Kern, R., and Varjú, D. (1998). Visual position stabilization in the hummingbird hawk moth, *Macroglossum stellatarum* L. I. Behavioural analysis. J. Comp. Phys. A 182, 225–237. doi:10.1007/s003590050173.
- Kleiner, M., Brainard, D., and Pelli, D. (2007). What's new in Psychtoolbox-3? *Perception 36 ECVP Abstract Supplement*.
- Krapp, H. G., and Hengstenberg, R. (1996). Estimation of self-motion by optic flow processing in single visual interneurons. *Nature* 384, 463–466.
- Lappe, M., Bremmer, F., and Van den Berg, A. V. (1999). Perception of self-motion from visual flow. *Trends in Cognitive Sciences* 3, 329–336.
- Lee, D. N. (1980). The optic flow field: the foundation of vision. *Phil. Trans. R. Soc. B* 290, 169–179.
- Lee, D. N., and Aronson, E. (1974). Visual proprioceptive control of standing in human infants. *Perception & Psychophysics* 15, 529–532.
- Lee, D. N., Davies, M. N. O., Green, P. R., and Van der Weel, F. R. (1993). Visual control of velocity of approach by pigeons when landing. *J. Exp. Biol.* 180, 85–104.

- Lee, D. N., and Lishman, R. (1977). Visual control of locomotion. *Scandinavian Journal of Psychology* 18, 224–230.
- Lee, D. N., and Reddish, P. E. (1981). Plummeting gannets: a paradigm of ecological optics. *Nature* 293, 293–294.
- Lee, D. N., Reddish, P. E., and Rand, D. T. (1991). Aerial docking by hummingbirds. *Naturwissenschaften* 78, 526–527.
- Lestienne, F., Soechting, J., and Berthoz, A. (1977). Postural readjustments induced by linear motion of visual scenes. *Exp. Brain Res.* 28, 363–384.
- Linander, N., Baird, E., and Dacke, M. (2016). Bumblebee flight performance in environments of different proximity. J. Comp. Phys. A 202, 97–103. doi:10.1007/s00359-015-1055-y.
- Lisney, T. J., Wylie, D. R., Kolominsky, J., and Iwaniuk, A. N. (2015). Eye morphology and retinal topography in hummingbirds (Trochilidae: Aves). *Brain Behav. Evol.*, 1–15. doi:10.1159/000441834.
- Logan, D., Kiemel, T., and Jeka, J. J. (2014). Asymmetric sensory reweighting in human upright stance. *PLoS ONE* 9, e100418.
- Martin, G. R. (2007). Visual fields and their functions in birds. *Journal of Ornithology* 148, 547–562. doi:10.1007/s10336-007-0213-6.
- Martin, G. R. (2009). What is binocular vision for? A birds' eye view. *Journal of Vision* 9, 14–14. doi:10.1167/9.11.14.
- Martinoya, C., and Delius, J. D. (1990). Perception of rotating spiral patterns by pigeons. *Biological Cybernetics* 63, 127–134.
- Masseck, O., Röll, B., and Hoffmann, K.-P. (2008). The optokinetic reaction in foveate and afoveate geckos. *Vision Res.* 48, 765–772. doi:10.1016/j.visres.2007.12.004.
- Mathieu-Costello, O., Suarez, R. K., and Hochachka, P. W. (1992). Capillary-to-fiber geometry and mitochondrial density in hummingbird flight muscle. *Resp. Physiol.* 89, 113–132.
- Maurice, M. (2006). Influence of the behavioural context on the optocollic reflex (OCR) in pigeons (*Columba livia*). J. Exp. Biol. 209, 292–301. doi:10.1242/jeb.02005.
- McArthur, K. L., and Dickman, J. D. (2011). State-dependent sensorimotor processing: gaze and posture stability during simulated flight in birds. *J. Neurophys.* 105, 1689–1700.
- Morgan, B., and Frost, B. J. (1981). Visual response characteristics of neurons in nucleus of basal optic root of pigeons. *Experimental Brain Research* 42, 181–188.
- Mronz, M., and Lehmann, F. O. (2008). The free-flight response of *Drosophila* to motion of the visual environment. *J. Exp. Biol.* 211, 2026–2045.
- Nakayama, K., and Loomis, J. M. (1974). Optical velocity patterns, velocity-sensitive neurons, and space perception: a hypothesis. *Perception* 3, 63–80.
- O'Carroll, D. C., Bidweii, N. J., Laughlin, S. B., and Warrant, E. J. (1996). Insect motion detectors matched to visual ecology. *Nature* 382, 63–66. doi:10.1038/382063a0.
- Ödeen, A., and Håstad, O. (2010). Pollinating birds differ in spectral sensitivity. J. Comp. Phys. A 196, 91–96.
- Oie, K. S., Kiemel, T., and Jeka, J. J. (2002). Multisensory fusion: simultaneous re-weighting of vision and touch for the control of human posture. *Cogn. Brain Res.* 14, 164–176.
- Palmisano, S., Allison, R. S., Schira, M. M., and Barry, R. J. (2015). Future challenges for vection research: definitions, functional significance, measures, and neural bases. *Frontiers in Psychology* 6. doi:10.3389/fpsyg.2015.00193.
- Pelah, A., and Barlow, H. B. (1996). Visual illusion from running. Nature 381, 283.
- Pelli, D. G. (1997). The VideoToolbox software for visual psychophysics: transforming numbers into movies. *Spatial Vision* 10, 437–442.
- Pete, A. E., Kress, D., Dimitrov, M. A., and Lentink, D. (2015). The role of passive avian head stabilization in flapping flight. *Journal of The Royal Society Interface* 12, 20150508. doi:10.1098/rsif.2015.0508.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team (2016). *nlme: linear and nonlinear mixed effects models*. Available at: http://CRAN.R-project.org/package=nlme.
- Previc, F. H., and Mullen, T. J. (1990). A comparison of the latencies of induced postural change and self-motion perception. *J. Vestib. Res.* 1, 317–323.
- R Core Team (2015). *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing Available at: http://www.R-project.org/.
- Ravi, S., Crall, J. D., McNeilly, L., Gagliardi, S. F., Biewener, A. A., and Combes, S. A. (2015). Hummingbird flight stability and control in freestream turbulent winds. *J. Exp. Biol.* 218, 1444–1452. doi:10.1242/jeb.114553.
- Read, T. J. G., Segre, P. S., Middleton, K. M., and Altshuler, D. L. (2016). Hummingbirds control turning velocity using body orientation and turning radius using asymmetrical wingbeat kinematics. J. R. Soc. Interface. doi:10.1098/rsif.2016.0110.
- Reichardt, W. (1986). Processing of optical information by the visual system of the fly. *Vision Res.* 26, 113–126.
- Reichardt, W. (1987). Computation of optical motion by movement detectors. *Biophysical Chemistry* 26, 263–278.

- Reichardt, W., and Wenking, H. (1969). Optical detection and fixation of objects by fixed flying flies. *Naturwissenschaften* 56, 424.
- Rico-Guevara, A., Fan, T.-H., and Rubega, M. A. (2015). Hummingbird tongues are elastic micropumps. *Proc. R. Soc. B* 282, 20151014. doi:10.1098/rspb.2015.1014.
- Rico-Guevara, A., and Rubega, M. A. (2011). The hummingbird tongue is a fluid trap, not a capillary tube. *PNAS* 108, 9356–9360.
- Ros, I. G., and Biewener, A. A. (2016). Optic flow stabilizes flight in ruby-throated hummingbirds. J. Exp. Biol., jeb.128488. doi:10.1242/jeb.128488.
- Sapir, N., and Dudley, R. (2012). Backward flight in hummingbirds employs unique kinematic adjustments and entails low metabolic cost. J. Exp. Biol. 215, 3603–3611.
- Schiffner, I., and Srinivasan, M. V. (2015). Direct evidence for vision-based control of flight speed in budgerigars. *Sci. Reports* 5, 10992. doi:10.1038/srep10992.
- Scholtyssek, C., Dacke, M., Kroeger, R., and Baird, E. (2014). Control of self-motion in dynamic fluids: fish do it differently from bees. *Biology Letters* 10, 1–4.
- Shaw, E., and Tucker, A. (1965). The optomotor reaction of schooling Carangid fishes. *Anim. Behav.* 13, 330–337.
- signal developers (2013). *signal: signal processing*. Available at: http://r-forge.r-project.org/projects/signal/.
- Soechting, J. F., and Berthoz, A. (1979). Dynamic role of vision in the control of posture in man. *Exp. Brain Res.* 36, 551–561.
- Sponberg, S., Dyhr, J. P., Hall, R. W., and Daniel, T. L. (2015). Luminance-dependent visual processing enables moth flight in low light. *Science* 348, 1245–1248. doi:10.1126/science.aaa3042.
- Sprayberry, J. D. H., and Daniel, T. L. (2007). Flower tracking in hawkmoths: behavior and energetics. *J. Exp. Biol.* 210, 37–45.
- Srinivasan, M. V., Lehrer, M., Kirchner, W. H., and Zhang, S. W. (1991). Range perception through apparent image speed in freely flying honeybees. *Visual Neuroscience* 6, 519– 535.
- Srinivasan, M. V., Poteser, M., and Kral, K. (1999). Motion detection in insect orientation and navigation. *Vision Res.* 39, 2749–2766.
- Srinivasan, M. V., Zhang, S., and Bidwell, N. (1997). Visually mediated odometry in honeybees. *J. Exp. Biol.* 200, 2513–2522.

- Srinivasan, M. V., Zhang, S., Lehrer, M., and Collett, T. (1996). Honeybee navigation en route to the goal: visual flight control and odometry. J. Exp. Biol. 199, 237–244.
- Stein, B. A. (1992). Sicklebill hummingbirds, ants, and flowers. *BioScience* 42, 27–33.
- Stoffregen, T. A. (1985). Flow structure versus retinal location in the optical control of stance. J. *Exp. Psych: Human Perception and Performance* 11, 554–565.
- Straw, A. D. (2008). Vision Egg: an open-source library for realtime visual stimulus generation. *Front. Neuroinform.* 2. doi:10.3389/neuro.11.004.2008.
- Straw, A. D., Branson, K., Neumann, T. R., and Dickinson, M. H. (2011). Multi-camera realtime three-dimensional tracking of multiple flying animals. *Journal of the Royal Society Interface* 8, 395–409.
- Straw, A. D., Rainsford, T., and O'Carroll, D. C. (2008). Contrast sensitivity of insect motion detectors to natural images. *Journal of Vision* 8. doi:10.1167/8.3.32.
- Strother, J. A., Nern, A., and Reiser, M. B. (2014). Direct observation of ON and OFF pathways in the *Drosophila* visual system. *Current Biology* 24, 976–983. doi:10.1016/j.cub.2014.03.017.
- Suarez, R. K., Lighton, J. R. B., Brown, G. S., and Mathieu-Costello, O. (1991). Mitochondrial respiration in hummingbird flight muscles. *PNAS*, 4870–4873.
- Suarez, R. K., Lighton, J. R. B., Moyes, C. D., Brown, G. S., Gass, C. L., and Hochachka, P. W. (1990). Fuel selection in rufous hummingbirds: ecological implications of metabolic biochemistry. *PNAS* 87, 9207–9210.
- Sun, H., and Frost, B. J. (1998). Computation of different optical variables of looming objects in pigeon nucleus rotundus neurons. *Nature Neuroscience* 1, 296–303.
- Tammero, L. F., and Dickinson, M. H. (2002a). Collision-avoidance and landing responses are mediated by separate pathways in the fruit fly, *Drosophila melanogaster*. J. Exp. Biol. 205, 2785–2798.
- Tammero, L. F., and Dickinson, M. H. (2002b). The influence of visual landscape on the free flight behavior of the fruit fly *Drosophila melanogaster*. *Journal of Experimental Biology* 205, 327–343.
- Tauber, E. S., and Atkin, A. (1968). Optomotor responses to monocular stimulation: relation to visual system organization. *Science* 160, 1365–1367.
- Taylor, G. K., and Krapp, H. G. (2007). Sensory systems and flight stability: what do insects measure and why? *Advances in Insect Physiology* 34, 231–316.
- Telford, L., and Frost, B. J. (1993). Factors affecting the onset and magnitude of linear vection. *Perception & Psychophysics* 53, 682–692.

- Tobalske, B. W., Biewener, A. A., Warrick, D. R., Hedrick, T. L., and Powers, D. R. (2010). Effects of flight speed upon muscle activity in hummingbirds. *J. Exp. Biol.* 213, 2515–2523.
- Ulanovsky, N., and Moss, C. F. (2007). Hippocampal cellular and network activity in freely moving echolocating bats. *Nature Neuroscience* 10, 224–233.
- Vo, H. D., Schiffner, I., and Srinivasan, M. V. (2016). Anticipatory manoeuvres in bird flight. *Sci. Reports* 6, 27591. doi:10.1038/srep27591.
- Ward, B. J., Day, L. B., Wilkening, S. R., Wylie, D. R. W., Saucier, D. M., and Iwaniuk, A. N. (2012). Hummingbirds have a greatly enlarged hippocampal formation. *Biol. Lett.* 8, 657–659.
- Wasserman, S. M., Aptekar, J. W., Lu, P., Nguyen, J., Wang, A. L., Keles, M. F., et al. (2015). Olfactory neuromodulation of motion vision circuitry in *Drosophila*. *Curr. Biol.* 25, 467– 472. doi:10.1016/j.cub.2014.12.012.
- Welch, K. C., Bakken, B. H., del Rio, C. M., and Suarez, R. K. (2006). Hummingbirds fuel hovering flight with newly ingested sugar. *PBZ* 79, 1082–1087.
- Wells, D. J. (1993). Muscle performance in hovering hummingbirds. J. Exp. Biol. 178, 39-57.
- Wertz, A., Gaub, B., Plett, J., Haag, J., and Borst, A. (2009). Robust coding of ego-motion in descending neurons of the fly. J. Neurosci. 29, 14993–15000.
- Wickham, H. (2009). ggplot2: elegant graphics for data analysis. Springer-Verlag New York.
- Willis, M. A., Avondet, J. L., and Zheng, E. (2011). The role of vision in odor-plume tracking by walking and flying insects. *J. Exp. Biol.* 214, 4121–4132.
- Winship, I. R., Hurd, P. L., and Wylie, D. R. W. (2005). Spatiotemporal tuning of optic flow inputs to the vestibulocerebellum in pigeons: differences between mossy and climbing fiber pathways. J. Neurophys. 93, 1266–1277. doi:10.1152/jn.00815.2004.
- Winterson, B. J., and Brauth, S. E. (1985). Direction-selective single units in the nucleus lentiformis mesencephali of the pigeon (*Columba livia*). *Experimental Brain Research* 60, 215–226.
- Wolff, D. (2006). Nectar sugar composition and volumes of 47 species of Gentianales from a southern Ecuadorian montane forest. *Annals of Botany* 97, 767–777.
- Wood, C. A. (1917). *The fundus oculi of birds especially as viewed by the ophthalmoscope*. Chicago: The Lakeside Press.
- Wylie, D. R. (2013). Processing of visual signals related to self-motion in the cerebellum of pigeons. *Frontiers in Behavioral Neuroscience* 7. doi:10.3389/fnbeh.2013.00004.

- Wylie, D. R., Gutiérrez-Ibáñez, C., and Iwaniuk, A. N. (2015). Integrating brain, behavior, and phylogeny to understand the evolution of sensory systems in birds. *Frontiers in Neuroscience* 9. doi:10.3389/fnins.2015.00281.
- Wylie, D. R. W., Bischof, W. F., and Frost, B. J. (1998). Common reference frame for neural coding of translational and rotational optic flow. *Nature* 392, 278–282.
- Wylie, D. R. W., and Crowder, N. A. (2000). Spatiotemporal properties of fast and slow neurons in the pretectal nucleus lentiformis mesencephali in pigeons. *J. Neurophys.* 84, 2529–2540.
- Wylie, D. R. W., and Frost, B. J. (1990). The visual response properties of neurons in the nucleus of the basal optic root of the pigeon: a quantitative analysis. *Experimental Brain Research* 82, 327–336.
- Wylie, D. R. W., and Frost, B. J. (1996). The pigeon optokinetic system: visual input in extraocular muscle coordinates. *Vis. Neurosci.* 13, 945–953. doi:10.1017/S0952523800009172.
- Wylie, D. R. W., and Frost, B. J. (1999). Responses of neurons in the nucleus of the basal optic root to translational and rotational flowfields. *J. Neurophys.* 81, 267–276.
- Wylie, D. R. W., Gutierrez-Ibanez, C., Pakan, J. M. P., and Iwaniuk, A. N. (2009). The optic tectum of birds: mapping our way to understanding visual processing. *Can. J. Exp. Psych.* 63, 328–338.
- Yartsev, M. M., and Ulanovsky, N. (2013). Representation of three-dimensional space in the hippocampus of flying bats. *Science* 340, 367–372.
- Yorzinski, J. L., Patricelli, G. L., Platt, M. L., and Land, M. F. (2015). Eye and head movements shape gaze shifts in Indian peafowl. J. Exp. Biol. 218, 3771–3776. doi:10.1242/jeb.129544.
- Yorzinski, J. L., and Platt, M. L. (2013). Selective attention in peacocks during predator detection. Anim. Cogn. doi:10.1007/s10071-013-0708-x.
- Zanker, J. M., Srinivasan, M. V., and Egelhaaf, M. (1999). Speed tuning in elementary motion detectors of the correlation type. *Biological Cybernetics* 80, 109–116.
- Zeigler, H. P., and Bischof, H.-J. (1993). Vision, brain, and behavior in birds. MIT Press.
- Zeil, J., and Wittmann, D. (1989). Visually controlled station-keeping by hovering guard bees of *Trigona (Tetragonisca) angustula* (Apidae, Meliponinae). J. Comp. Phys. A 165, 711– 718.