A comparative study of gaze stabilisation in Dipteran flies

Ben Hardcastle

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Imperial College London
Department of Bioengineering

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Declaration

I hereby declare that the work presented in this thesis is entirely my own, with the exception of the collection of experimental data and video processing, which was partly carried out in collaboration with laboratory technician Karin Bierig. Where others have contributed, every effort has been made to accurately reference and acknowledge their work.
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Abstract

Flying insects, like many other animals that rely on their sense of vision to guide behaviour, have a tendency to maintain a default orientation of their eyes relative to the environment. During flight, reflexes act to keep the head level and minimise retinal image shifts resulting from rotational steering manoeuvres, or from external perturbations such as wind gusts and turbulent air flow. Gaze stabilisation serves a number of functions, which include: i) simplifying the estimation of translational self-motion, ii) aligning the head-based sensory systems with the inertial vector which facilitates the transformation of sensory signals into motor commands, iii) supporting the tracking of moving targets, and iv) reducing motion blur in the visual input.

This thesis reports studies on species-specific adaptations and general principles underlying multisensory gaze stabilisation in a number of different Dipteran flies. A variety of stimulation methods were explored, along with their suitability for a linear systems analysis of the gaze stabilisation system across species. Using results obtained from the well-characterised blowfly for comparison, novel experimental work was performed on the gaze stabilisation behaviour of robberflies, hoverflies and horseflies. Species from each family were shown to stabilise their heads in compensation for body rotations around the roll axis. The performance of the reflex was found to be species-specific and dependent on the sensory modalities involved. Experimental evidence suggests that in contrast to the other families, hoverflies appear to make use of the inertia of the head to maintain a level gaze, a novel finding that has previously been reported only for dragonflies. Finally, the integration of signals in the context of gaze stabilisation obtained by the two visual systems in blowflies—the ocelli and compound eyes—were explored in both behavioural and electrophysiological experiments.

This research opens new lines of investigation by identifying behaviours that demonstrate different control strategies employed by the nervous systems of flying insects.
# Table of contents

1. Introduction ............................... 9
   1.1 Overview ........................................ 9
   1.2 Behavioural studies in insects .............. 10
   1.3 Gaze stabilisation ............................. 12
      1.3.1 Gaze stabilisation in flies .............. 13
      1.3.2 Compound eyes ............................ 14
      1.3.3 Ocelli ..................................... 15
      1.3.4 Halteres .................................. 16
      1.3.5 Lobula plate tangential cells .......... 17
      1.3.6 Variation in Dipteran LPTCs .......... 20
      1.3.7 Species studied ........................... 20
      1.3.8 Roll stabilisation and sensing ....... 23

2. Behavioural paradigms for investigating gaze stabilisation 25
   2.1 Introduction ................................... 25
   2.2 Materials and methods ....................... 27
      2.2.1 Animal preparation ....................... 27
      2.2.2 Equipment .................................. 27
      2.2.3 Conventions ................................ 29
      2.2.4 Video analysis ............................. 29
      2.2.5 Conditions .................................. 29
   2.3 Chirp stimulus ............................... 30
      2.3.1 Design and implementation ............... 30
      2.3.2 Results .................................... 32
      2.3.3 Slip speed increases in the absence of haltere input .. 33
      2.3.4 Gain and phase ............................ 34
      2.3.5 Comparison with constant-frequency sinusoidal stimuli ... 37
      2.3.6 Discussion .................................. 38
   2.4 Random-noise stimulus ...................... 39
      2.4.1 Design and implementation ............... 39
      2.4.2 Results .................................... 40
      2.4.3 Discussion .................................. 43
   2.5 Concluding remarks ........................... 44

3. Head-roll stabilisation in three Dipteran families 46
   3.1 Horsefly ........................................ 46
      3.1.1 Introduction .................................. 46
      3.1.2 Materials and methods ........................ 47
      3.1.3 Results ...................................... 47
      3.1.4 Significance of haltere input ............ 50
      3.1.5 Discussion .................................. 52
   3.2 Hoverfly ........................................ 55
      3.2.1 Introduction .................................. 55
      3.2.2 Initial behavioural observations ........ 56
      3.2.3 Chirp response, Episyphus balteatus .. 56
      3.2.4 Further behavioural observations ........ 58
      3.2.5 Frequency response, Eristalinus aeneus .... 59
      3.2.6 CFS frequency response, Eristalinus aeneus . 60
      3.2.7 Retinal slip speeds ........................ 62
3.2.8 Discussion ................................................................. 63
3.3 Robberfly ................................................................. 67
  3.3.1 Introduction ......................................................... 67
  3.3.2 Roll behaviour experiments .................................... 67
  3.3.3 Frequency response ............................................... 68
  3.3.4 Ocelli effect gain and phase ..................................... 70
  3.3.5 Discussion .......................................................... 70

4. Integration of visual pathways in the blowfly  72
  4.1 Introduction .......................................................... 72
  4.2 Materials and methods .............................................. 73
    4.2.1 Behavioural experiments .................................... 73
    4.2.2 Linear feedback model ....................................... 73
    4.2.3 Electrophysiology ............................................. 75
  4.3 Behavioural results .................................................. 76
    4.3.1 Blowfly head-roll response .................................. 76
    4.3.2 Blowfly frequency response .................................. 77
    4.3.3 Individual pathway responses ................................ 79
    4.3.4 Robberfly head-roll behaviour ................................ 81
  4.4 Interneuron electrophysiology .................................... 83
    4.4.1 VS and DNOVS responses ..................................... 84
  4.5 Discussion ............................................................ 87
    4.5.1 Ocellar effect on head-roll delay and amplitude ........ 87
    4.5.2 Behavioural results indicate non-linearity ................. 89

5. Concluding remarks  92
  5.1 Summary ............................................................. 92
  5.2 Future directions .................................................... 96

Bibliography  98
List of figures and tables

Figure 1.1: Gaze stabilisation in response to forced thorax-roll.......................... 13
Figure 1.2: Dorsal ocelli of the blowfly .............................................................. 15
Figure 1.3: Halteres of the blowfly................................................................. 16
Figure 1.4: Schematic of the fly optic lobe ......................................................... 17
Figure 1.5: VS cells of the blowfly ................................................................. 18
Table 1.1: Summary of fly families ............................................................... 21
Figure 1.6: Roll turns of the blowfly during flight ........................................ 24

Figure 2.1: Setup of behavioural experiments ............................................. 28
Figure 2.2: Chirp stimulus spectrogram .......................................................... 31
Figure 2.3: Mean responses to chirp stimulus .................................................. 33
Figure 2.4: Distribution of slip speeds in response to chirp stimulus .......... 34
Figure 2.5: Relative head-roll gain and phase in response to chirp stimulus .... 35
Figure 2.6: Comparison of head-roll gain and phase for chirp and CFS stimuli ... 37
Figure 2.7: Bandwidth-limited noise stimulus .............................................. 39
Figure 2.8: Mean head-roll response to random-noise stimulus ................... 41
Figure 2.9: Gain and slip speeds in response to noise stimulus .................... 42

Figure 3.1: Image of the horsefly, Tabanus bromius ...................................... 47
Figure 3.2: Frequency response of the horsefly head to a thorax-roll rotation ...... 48
Figure 3.3: Retinal slip speed distribution during CFS thorax-roll experiments .. 51
Figure 3.4: Median slip speed during CFS thorax-roll experiments .................. 51
Figure 3.5: Mean chirp response traces .......................................................... 57
Figure 3.6: Head movements of hoverfly E. balteatus during flight ............... 58
Figure 3.7: Images of the hoverflies, E. tenax and E. aeneus ......................... 59
Figure 3.8: Frequency response of E. aeneus head-roll with chirp stimulus ...... 61
Figure 3.9: Frequency response of E. aeneus head-roll with CFS stimuli .......... 61
Figure 3.10: Retinal slip speed in the hoverfly E. aeneus ............................... 62
Figure 3.11: Model response to chirp stimulus .............................................. 66
Figure 3.12: Image of the robberfly, Philonicus albiceps ............................... 68
Figure 3.13: Frequency response of the robberfly head to thorax-roll rotation .... 69

Figure 4.1: Linear feedback model ............................................................... 74
Figure 4.2: Setup of electrophysiology experiment ....................................... 76
Figure 4.3: Blowfly head-roll response traces ................................................ 77
Figure 4.4: Blowfly frequency response of the head to roll of visual horizon ...... 78
Table 4.1: Head-roll response delay attributed to the ocelli ............................ 78
Figure 4.5: Open-loop frequency responses .................................................. 80
Figure 4.6: Robberfly head-roll response traces ............................................ 82
Figure 4.7: Robberfly frequency response of the head to roll of visual horizon ... 82
Figure 4.8: Summary of neuroanatomy .......................................................... 84
Figure 4.9: VS and DNOVS responses ........................................................... 85
Figure 4.10: Normalised VS and DNOVS responses ...................................... 86

Figure 5.1: Phylogenetic relationships between Diptera .................................. 94
Figure 5.2: Frequency response of all flies tested ......................................... 94
1. Introduction

1.1 Overview

For neuroethologists, insects are a useful model system because of their accessibility, the ease of their study—both in the field and in culture in the laboratory—and for having relatively simple nervous systems which have evolved elegant solutions for performing often complex behavioural tasks. As a result, fundamental principles of visual processing, sensory feedback control and multisensory integration have been successfully identified in insects (Sterling and Laughlin, 2015). Dipteran (‘two-winged’) flies, such as fruitflies and blowflies, have offered valuable insight into sensorimotor control—the process that links state estimates based on sensory inputs to a coordinated activation of the various motor systems enabling a robust behavioural output. Sensorimotor reflexes in flies have evolved to facilitate rapid flight control at a level of performance that is unmatched by current man-made aerial devices. Understanding the functional principle underlying these reflexes in flies could potentially inspire the design of control architectures supporting autonomous, energy-efficient and high performance micro-air vehicles (MAVs).

An important sensorimotor control loop for many animals stabilises the eyes with respect to the environment during locomotion. Previous work on this gaze stabilisation system in flies has primarily focussed on blowflies and fruitflies, and some of the insights gained have found application in engineered systems such as MAVs (Kerhuel et al., 2007; Gremillion et al., 2014). However, Dipteran flies are diverse and adaptable. They are found in almost every habitat on land and display a wide range of behaviours, flight styles, body shapes, and sizes, so the commonly studied species are hardly typical.

The general organization of neuropils responsible for the early stages of visual processing appear to be conserved across Diptera (Buschbeck and Strausfeld, 1996).
The higher level processing, however, which ultimately transforms visual information into appropriate commands for gaze stabilisation in addition to locomotor control, involves a set of identified neurons known as the lobula plate tangential cells (LPTCs); these neurons respond to the optic flow induced by self-motion, and their morphology has been shown to vary widely between closely related species (Krapp and Hengstenberg, 1996; Buschbeck and Strausfeld, 1997). Furthermore, the dynamic response properties of LPTCs have been suggested to correlate with species-specific flight dynamics across different families of flying insects (O’Carroll et al., 1996).

This variation in cellular morphology and physiological response properties gives rise to the question of whether or not the gaze stabilisation behaviour similarly varies between species. If so, there may be adaptations of individual sensors, the processing and integration of their signals, or the specific strategies employed to support the natural behaviour of each species. These are the areas this work seeks to investigate. By uncovering such specialisations, the commonalities may also be revealed, thereby illustrating the general principles of gaze stabilisation and how the nervous system is related to the body and behaviour.

1.2 Behavioural studies in insects

A commonly held assumption is that insect brains are deterministic, “simple reflex machines” (Chittka and Gordon, 2016), and that it is possible to predict their behaviour based on external stimuli alone. This has recently been challenged by evidence for selective attention within insect visual systems (Wiederman and O’Carroll, 2013; Paulk et al., 2014), and internal modulation of neural processing (Maimon et al., 2010), via release of neurotransmitters during different locomotor states, nutritional states or wakefulness states (Andretic et al., 2005; Suver et al., 2012; Longden et al., 2014). These findings bring a new dimension to the complexity of behavioural
1. Introduction

studies and may require additional experimental controls to be performed. The nervous systems of insects are, however, less complex in terms of quantity of neurons than those of typical mammalian model systems studied in neuroscience (Strausfeld, 1976), and in aspects of visually guided sensorimotor control, such as flight, their behaviour can be considered largely deterministic (Censi et al., 2013). In several cases, it has been possible to identify subsets of neurons, or even single neurons, present in each individual of a given species, which play a significant role in a particular behaviour. An example within the visual system is the detection of looming objects which indicate an imminent collision: this appears to be a common feature across insect visual systems, often attributed to the response properties of an individually identified cell (fly: (De Vries and Clandinin, 2012); locust: (Rowell et al., 1977; Rind, 1987; Peron et al., 2009); moth: (Collett, 1972; Wicklein and Strausfeld, 2000)).

This potentially allows an entire sensorimotor loop to be examined: from the sensory detection, through processing stages in identified neurons, to the behavioural output via muscles and limbs. With the toolbox of genetic manipulations available for the fruitfly, Drosophila melanogaster, and the ability to monitor and control the activity of the nervous system on an unprecedented scale, whole-system studies have become possible (Pfeiffer et al., 2008; Behnia and Desplan, 2015; Ohyama et al., 2015). This leverage of the insect nervous system ideally positions it for understanding the mechanisms of wide-reaching effects, such as state-dependent processing of sensory information. The advent of CRISPR systems may accelerate the application of similar techniques to other insects (review: Wright et al., 2016), but for the time being, the comparative study of species-specific adaptations is an area where genetic manipulations are not necessarily required.
1.3 Gaze stabilisation

The task of stabilising gaze is performed by many visual animals. Tadpoles, crabs, owls, flies, and primates, with their widely varying eye designs, all employ strategies to minimise retinal slip—the motion of images across the eye (Money and Correia, 1972; Keller, 1978; Paul et al., 1990; Miles and Wallman, 1993; Sandini et al., 2001; Lambert et al., 2012). The processing of visual information is energetically expensive, and the evolution across phyla of a reflex stabilising the eyes suggests that it is a worthwhile investment to preserve the fidelity of the information obtained by vision (Laughlin et al., 1998). One key benefit of stabilising gaze is to avoid blurring and a reduction of spatial resolution, caused by relative motion due to locomotion, movement of objects in the environment, or a combination of both. Visually guided behaviours rely on accurate information about spatial orientation (Angelaki and Hess, 2005), and an animal’s ability to accurately perceive image details decreases with increasing speed of motion (Land, 1999). Gaze stabilisation reduces image motion as an animal moves within its environment, thus preserving spatial resolution.

Translational visual information is useful for determining object distance and velocity through motion parallax (Angelaki and Hess, 2005). Translational optic flow information can be corrupted by rotational components, leading to ambiguities; gaze stabilisation often reduces these rotational movements. The neural processing of visual information is also simplified when the eyes are stabilised relative to the orientation of the environment and, in addition, this can facilitate the detection of moving figures against the background (Land, 1999).

The eyes also move to actively sample visual features relevant to the task at hand (Yarbus, 1967; Land, 1999), and when rotations of the head or body are made under voluntary control, the eyes may move to the new orientation first, or move more quickly than the head and body, so as to minimise the time in which blurring can occur (Schilstra and van Hateren, 1998; Kandel et al., 2000). However, the term
1. Introduction

‘gaze stabilisation’ is used in this work to describe reflexes in response to unanticipated external disturbances (review: Hardcastle and Krapp, 2016).

1.3.1 Gaze stabilisation in flies

In mammals, the vestibulo-ocular reflex (VOR) makes use of estimates of head rotation from the vestibular system to make compensatory movements of the eyes in the opposite direction to the head, while the optokinetic reflex (OKR) makes use of retinal slip to stabilise the eyes against the environment by moving them in the same direction as the visual motion (Kandel et al., 2000; Cullen, 2012). Since the eyes of the fly are fixed in position on the head, the two cannot be moved independently and gaze is therefore stabilised by movements of the head, but an analogy can be made to two reflexes observed in flies. The optomotor response initiates a yaw turn of the whole animal in the same direction as the movement of an external visual pattern (Poggio and Reichardt, 1976; Collett, 1980), much like the OKR.

![Figure 1.1: Gaze stabilisation in response to forced thorax-roll. A video frame from an experiment by Hengstenberg (1993) which shows the level head of the fly while its thorax is offset at an angle.](image)

Analogous to the VOR, rotation of the whole animal by external disturbances initiates compensatory movements of the head in the opposite direction to the body (review: Hengstenberg, 1993). This is a multisensory process which integrates information from sensors located on both the head and body. In doing so, estimates of the orientation of both head and body are obtained and, just as importantly, the
sensors have different response characteristics, which expands the operating range of the stabilisation system (Hengstenberg, 1991).

In the following sections, the sensory modalities studied in this project are introduced, and their functional properties and contributions to roll gaze stabilisation in the blowfly are presented. The sensors are categorised as either visual, which detect changes in light intensity, or mechanosensory, which detect changes in force.

1.3.2 Compound eyes

The compound eyes are the primary visual system of Dipteran flies. Composed of around 4,000 individual eye facets in blowflies, they have a panoramic field of view covering over 85% of their surroundings (Franz and Krapp, 2000; Petrowitz et al., 2000). They have relatively good spatial acuity compared to other insects and are critical for flight control and navigation (Land and Nilsson, 2002). They contribute to gaze stabilisation over a low-mid range of motion velocities; Hengstenberg found that with input from the compound eyes alone, the amplitude of compensatory head-roll movement peaks at an angular velocity of around 70°/s in blowflies (1984).

Neuronal responses from the photoreceptors of the compound eye pass through several layers of processing before behavioural output is modified. Response times of the gaze stabilisation reflex, when mediated by the compound eyes alone, are around 30 ms in blowflies, slow in comparison to the response of other modalities such as the halteres (Hengstenberg, 1993). Signals from the compound eyes are processed retinotopically in separate columns throughout the optic lobes of the brain (Borst, 2009). Wide-field patterns of motion are extracted by the tangential cells in the third visual neuropil, which may be used by the gaze stabilisation and flight control systems—these are discussed further in Section 1.3.5. Descending neurons then integrate the signals from the tangential cells and other sensory modalities, including the secondary visual system, the ocelli (Strausfeld and Bassemir, 1985; Haag et al., 2007).
1.3.3 Ocelli

The ocelli of the blowfly are three individual lens eyes situated dorsally on the head between the two compound eyes. The lenses do not form a focussed image on their photoreceptor units, so spatial acuity is low (Land and Nilsson, 2002). They are highly sensitive to changing light levels though, and with a field of view which extends to the horizon, their postulated function is to aid orientation by discriminating between the sky and ground (Schuppe and Hengstenberg, 1993). They have been shown to support a transient dorsal light reflex, in combination with the compound eyes, which orients the dorsal side of the fly towards the centre of brightness in its surroundings. They respond more quickly than the compound eyes, with latencies of around 6 ms, and appear to provide a rapid, but imprecise, estimate of attitude changes. The role of the ocelli in gaze stabilisation remains unclear, and is examined more closely in Chapter 4.

Figure 1.2: Dorsal ocelli of the blowfly. The horsefly studied in Chapter 3 lacks this system of three eyes.
1. Introduction

1.3.4 Halteres

The halteres are a mechanosensory system involved in gaze stabilisation and flight control. Once functional hind-wings, as in four-winged insects, they have evolved into small, club-shaped structures which beat in anti-phase with the front wings.

The gyroscopic effect of the beating halteres resists changes to their plane of motion (Chan et al., 1998), and strain sensors at their base, known as campaniform sensilla, detect Coriolis forces caused by rotations of the thorax in flight (Nalbach, 1993). The haltere afferent fibres synapse directly with neck motor neurons to provide a low latency head-roll response within around 5 ms (Sandeman and Markl, 1980; Taylor and Krapp, 2007). They are sensitive to high angular velocities, and gaze and flight stabilisation are severely affected by their removal (Preuss and Hengstenberg, 1992); some neck motor neurons in the blowfly have been shown to only respond to visual stimuli when coincident with haltere signals (Huston and Krapp, 2009).

![Halteres diagram](image)

**Figure 1.3:** Halteres of the blowfly. Top, left: Position of the halteres. Bottom, left: The structure of the haltere, showing the distal knob on the left. Top, right: The amplitude of head-roll movements in response to rotations of the thorax, with no visual features, illustrating the range of velocities over which the halteres mediate gaze stabilisation. Bottom, right: The compound eye mediated head-roll in response to a patterned drum rotated around the fly shows maximum contributions in a complementary range of lower velocities. Modified from (Hengstenberg, 1993).
1. Introduction

Other mechanosensory systems consist of fields of sensitive hair cells that detect the angle between the head and thorax to correct static errors in orientation (Preuss and Hengstenberg, 1992; Taylor and Krapp, 2007). Their contributions to gaze stabilization are subtler and were not manipulated in the experiments described in Chapters 2–4, however, they are discussed further in the context of the findings in Section 3.2.8.

1.3.5 Lobula plate tangential cells

Pattern motion across the compound eye causes light intensity changes that are correlated both spatially and temporally. In each of the individual units of the compound eye, known as ommatidia, photoreceptors convert light intensity modulations into neural signals. These are amplified in the first layer of the optic lobe, the lamina, and then passed on to the second layer, the medulla (Strausfeld, 1976).

![Figure 1.4: Schematic of the fly optic lobe. The input dendrites of the VS cells, and other tangential cells (LPTCs), lie within the lobula plate. Modified from (Tuthill, 2012).](image)

Neural circuits hypothesised to be located in the medulla and the third neuropil, the lobula, extract the direction of motion across ommatidia according to the elementary motion detector (EMD) model first described by Hassenstein and Reichardt (1953; Borst et al., 2010). Local motion information is then input to the lobula plate with the retinotopic mapping conserved. The lobula plate tangential cells spatially pool the outputs from thousands of directionally selective EMDs. In this way,
the LPTCs receive input on their dendrites representing motion across large areas of the compound eye which, in some cases, also includes contralateral input (Taylor and Krapp, 2007).

Two subpopulations of LPTCs provide output from the lobula plate, the vertical and horizontal system (VS and HS) cells. ‘Vertical’ and ‘horizontal’ describe the orientation of the main dendrites of the cells, as well as the direction of motion they are predominantly sensitive to (Dvorak et al., 1975; Hausen, 1982a, 1982b; Hengstenberg et al., 1982). Examining the receptive fields of the VS cells more closely reveals that the preferred direction of local motion is not the same across the visual field. The pattern of their local preferred directions resembles the wide-field motion, or optic flow, generated by rotations of the entire visual field relative to the fly. It appears that the VS cells are tuned to detect optic flow generated by rotations around horizontal body axes (Koenderink and van Doorn, 1987; Krapp and Hengstenberg, 1996).

**Figure 1.5:** VS cells of the blowfly. Top: Morphology of 3 tangential cells in the lobula plate, based on intracellular stains. Bottom: Local motion preference, represented by vector direction, and sensitivity, represented by vector length, sampled throughout each cell’s visual receptive field. Modified from (Taylor and Krapp, 2007)
1. Introduction

Each VS cell functions as a matched filter to sense a particular rotatory self-motion (Franz and Krapp, 2000). The VS1 cell, for instance, monitors nose-up pitch; VS6 indicates roll; and VS8 responds most strongly to a rotation around an axis between pitch and roll (Figure 1.5).

The HS cells, on the other hand, appear to be tuned to optic flow around the vertical body axis, as generated by a rotation similar to yaw, and are also implicated in the discrimination of yaw rotations from forward translation through binocular interactions (Krapp et al., 2001). In a series of replay experiments—whereby a panoramic visual stimulus presented the optic flow recreated from a fly’s flight path in a physical arena—one of the HS cells, HSE, could be studied during simulated rotational and translational self-motion. The cell’s response during bouts of forward translation was found to vary with the size of the simulated arena, implying that the nervous system may have access to information about the spatial organisation of the insect’s surrounds, including distance information, through the HS cells (Kern et al., 2005).

The tangential cells are strongly implicated in the control of both gaze and flight (Dvorak et al., 1975; Hausen, 1976; Huston and Krapp, 2008; Haikala et al., 2013). Processing visual information in non-orthogonal rotation axes, rather than horizontal and vertical, may set up a coordinate system that is already tailored to the requirements of the neck motor system (Huston and Krapp, 2008), thus reducing the additional computation required to convert sensory signals into motor commands (Taylor and Zbikowski, 2005). The number of cells implies some redundancy, since any rotation could be encoded around three orthogonal axes. However, a greater number of cells enables more reliable responses, and lateral coupling between neighbouring cells extends the receptive field of each individual cell (Haag and Borst, 2004; Karmeier et al., 2005). It has also been hypothesised that the non-orthogonality of the system reflects the flight dynamics of the insect, and that the
LPTCs are tuned to detect potentially unstable natural modes of motion during flight (Krapp et al., 2012).

1.3.6 Variation in Dipteran LPTCs

Most of the knowledge of optic flow processing in LPTCs has been gained from electrophysiological work on blowflies. LPTCs are found in other flies, but have not been studied nearly as extensively. The anatomy of LPTCs in a wide range of Dipteran species was studied by Buschbeck and Strausfeld (1997). They found that the morphology of the retinotopic, small-field neurons in the medulla, which are partly responsible for motion detection pre-synaptic to the LPTCs, was phylogenetically conserved across distantly related species (Buschbeck and Strausfeld, 1996). In contrast, the lobula plate and the tangential neurons varies greatly. The number of cells, their distribution, depth and morphology were found to differ even in closely related species (Buschbeck and Strausfeld, 1997). In many species, a distinction could not be made between VS- and HS-type cells based on their morphology.

Based on these morphological differences among flies, they put forward the hypothesis that LPTCs have evolved to suit the behaviour of the animal. Specific styles of flight, such as hovering or fast saccades, would result in quite different patterns of optic flow. As the LPTCs sense optic flow to control flight and gaze stabilisation, it seems reasonable that they might be adapted to sense the most frequent or important patterns of self-motion. Visual ecology might therefore explain the divergence of LPTC morphology among Diptera, which may in turn be reflected in their gaze stabilisation behaviour.

1.3.7 Species studied

In this comparative study, species of fly were chosen with dissimilar flight behaviours, or with particular configurations of the LPTCs that contrast with the blowfly.
1. Introduction

The distinguishing characteristics of these flies are summarised in Table 1.1. From these generalised traits, a number of predictions can be made.

<table>
<thead>
<tr>
<th>Family (common name)</th>
<th>Archetypal body shape</th>
<th>Distinguishing flight behaviour</th>
<th>Differences with Calliphora</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calliphoridae</strong> (blowflies)</td>
<td>Short, stubby, round</td>
<td>Saccadic search</td>
<td>—</td>
</tr>
<tr>
<td><strong>Tabanidae</strong> (horseflies)</td>
<td>Wide, flat</td>
<td>Fast, straight flight</td>
<td>Lack of dorsal ocelli (simple eyes)</td>
</tr>
<tr>
<td><strong>Syrphidae</strong> (hoverflies)</td>
<td>Blowfly-like, or short slender tube</td>
<td>Hovering flight Saccadic search</td>
<td>Second complement of VS cells in some species¹</td>
</tr>
<tr>
<td><strong>Asilidae</strong> (robberflies)</td>
<td>Long, slender, tube</td>
<td>Aerial prey capture launched from perches</td>
<td>Lack of VS-like cells¹</td>
</tr>
</tbody>
</table>

Table 1.1: Summary of fly families. The simplified characteristics of flies from each of the families studied. These are generalisations which may be useful for reference. There are species within each family that do not fit these descriptions, and there are also many similarities that exist between the flies studied.¹Buschbeck and Strausfeld (1997)

Tabanids lack the fast visual input afforded by ocelli and have been observed to take mostly direct flight paths, rather than making sequences of rapid saccades. They are also generally larger in size than *Calliphora vicina*. As such, stabilising movements of the head made by horseflies may be slower and less agile than those seen in blowflies. Previous work on Syrphids has shown that they do not stabilise their gaze during yaw and roll turns in flight (Collett and Land, 1975; Schilstra, 1999), however the neural anatomy of the tangential cells in *Eristalis tenax*, for instance, shows remarkable similarity with the HS and VS cells of the blowfly, and some hoverfly species appear to have a duplicate set of VS-like cells, the purpose of which is still not known (Buschbeck and Strausfeld, 1997). For hovering, visual
feedback based on the detection of features such as the horizon must surely be crucial for the rapid posture control required to maintain an approximately static position. In this situation, it is possible that stabilisation may be carried out by the wings, rather than the neck, which would explain its absence in previous experiments. Yet, given the fast, forward-flight speeds of many species (Buschbeck and Strausfeld, 1997) and the acrobatic chasing exhibited during mating, it would be surprising if hoverflies did not also make compensatory head movements for some fast roll turns, to avoid significant motion blurring.

Asilids appear to be highly specialised for making short prey-capture flights in bright environments with direct sunlight, and have not been seen to sustain flight for long periods, like calliphorids or syrphids. Their heads are highly mobile, and have been observed making fast saccades while perched, presumably to bring potential aerial targets onto a particular region of the eyes. Their stabilisation behaviour during flight may depend on the strategy they employ to chase their prey. A pre-determined, ballistic interception course would require compensation for external perturbations and course-corrections relative to the environment in order to remain on-track—for this, an effective gaze stabilisation system as seen in blowflies would be required. Alternatively, a pursuit strategy using visual feedback of the target’s position would likely function by attempting to maintain the target within a certain region of the eyes or at a certain angle, and this may take priority over stabilisation relative to the environment. The computations required for tracking a small, moving visual object would, however, benefit from gaze stabilisation relative to the target’s path. Prey-capture flights and gaze stabilisation strategies in robberflies have not been studied, but an absence of LPTCs with the typical dendrites of VS-type cells, as seen in some robberfly species (Buschbeck and Strausfeld, 1997) could indicate a reduced need for stabilisation based on wide-field motion.
1.3.8 Roll stabilisation and sensing

Roll stabilisation has been chosen for study, rather than yaw stabilisation, for a number of reasons: firstly, the range of head movements is larger (roughly ±90° vs. ±20° for blowflies (Hengstenberg et al., 1986)) and a wider range therefore exists over which performance drops may be detectable with a video camera. Secondly, compensatory yaw head movements may be coupled with head-roll (Nalbach and Hengstenberg, 1994), which may complicate both the measurement of head movements and the interpretation of their causes. Related to this, yaw head movements may also be used by flies to direct their gaze towards an object or feature, independently of stabilisation reflexes. Roll head movements appear to be made only in support of stabilisation and should be more straightforward to interpret (head-roll may also be observed during grooming; however, this should be easily identifiable due to the involvement of the legs). Lastly, a wide body of existing work on roll stabilisation in the blowfly demonstrates the limits of the system and allows for a quantitative comparison of the performance under different experimental conditions. Against this, the performance of other species will be compared.

Sensing of roll rotations involves multiple sensory modalities, as described in the preceding sections. The dynamic ranges of each appear to complement each other, as illustrated in Figure 1.3—the sensitivity peak of the halteres is at a range of high roll velocities, which the visual systems do not appear to operate in. The ranges of the halteres and visual systems overlap somewhat, and the visual systems show greater sensitivity to low velocity roll. In this way, the overall sensing range may be extended. Schilstra and van Hateren (1999) showed that for blowflies flying in a small arena, the maximum roll angular velocities of the thorax were around 2000°/s, corresponding to a 15 Hz stimulus in the behavioural experiments performed herein. Sensing of rotations in this range could only be performed by the halteres; the velocity at which motion blur becomes an issue for the blowfly compound eye is an
1. Introduction

An order of magnitude lower than this, at around 200°/s (van Hateren, 1992). By making use of the haltere signal, an initial compensatory movement should reduce the velocity of the head to within a range that the visual system can operate, and Section 2.3.3 will show the effect that removing the halteres has on this control design.

Figure 1.6: Roll turns during flight. Left: Flight path of a typical manoeuvre made by a blowfly. Starting at 0, the fly’s position is drawn every 20 ms, with its front indicated by a filled circle and its heading indicated by the orientation of the line. Centre: The angular position (top) and velocity (bottom) of the fly’s thorax throughout the manoeuvre was analysed in terms of yaw, pitch and roll components. The highest roll angles and velocities (blue) made by the flies were observed during these turns. Right: Probability densities of angular velocities over 1781 seconds of flight by 10 flies. Modified from (Schilstra and van Hateren, 1999)

At least 90% of turns made by blowflies, as measured by Schilstra and van Hateren, involved roll angles of less than 30°, so a similar range is tested here, up to at least 15 Hz. The sensing range of the halteres, the visual systems, and other modalities which contribute to roll gaze stabilisation, may be tuned to the flight behaviour of a particular species. Hovering flies, for example, may require sensing of much smaller angular deviations or rotations over a short time-scale, associated with local turbulent airflow. A wide range of stimulus velocities is therefore used, spanning from approximately 1–2400°/s, in an attempt to capture the sensitivities of each species.
2. Behavioural paradigms for investigating gaze stabilisation

This chapter describes the methods used to investigate roll gaze stabilisation in tethered, flying flies. A methodology needed to be established as a framework for analysing the multisensory integration involved in roll stabilisation behaviour across species. In the first section, previous methods are discussed, which have largely made use of constant-frequency sinusoidal (CFS) oscillations as a stimulus. Section 2.3, presents an alternative ‘chirp’ stimulus with a frequency which continuously changes over time, allowing the frequency response to be obtained more quickly than with CFS stimuli. Section 2.4 presents a stimulus signal with a Gaussian distribution of angular velocities, to examine how the gaze stabilisation system responds to a bandwidth-limited random noise input. Both stimuli were tested on blowflies so that the findings could be interpreted in relation to the results from previous work.

2.1 Introduction

Blowflies display a powerful reflex which maintains a level gaze while the thorax manoeuvres during flight. Fixing a fly’s thorax to a stick and holding it aloft, with its wings free to flap, is sufficiently similar to the condition of actual flight that the gaze stabilisation reflex is activated when the fly is rotated around its longitudinal body axis. Various sensory modalities contribute input to this reflex (see Section 1.3.1), and much of what is known about their involvement has been learned from using a motor to oscillate the thorax of the fly in this tethered flight preparation.

A sinusoidal roll motion of ±30° is similar to the banked turns that blowflies make during flight (van Hateren and Schilstra, 1999), and with a static visual cue such as a horizon or grating pattern, mechanosensors and visual sensors are stimulated. In recent studies, systems identification techniques have been applied to understand the dynamics and interplay between different sensory modalities, and to
understand the sensorimotor control strategies employed in a biological organism from an engineering perspective (Theobald et al., 2010; Schwyn et al., 2011; Fuller et al., 2014; Goulard et al., 2015). Sinusoidal input stimuli have traditionally been used for linear systems analyses, including the study of gaze control reflexes; most notably for flies in the work by Hengstenberg (review: 1991), as well as for a variety of other insects (Barnes, 1993), amphibians (Dieringer and Precht, 1982), birds (Haque and Dickman, 2005) and mammals, including humans (Barnes, 1993).

Schwyn et al. (2011) showed via superposition of sinusoidal inputs that the gaze stabilisation reflex can be approximated by a linear system, concluding that despite the numerous individual non-linear processes involved, the behavioural output exhibits a high degree of linearity. The use of sinusoidal stimuli is suitable for an input-output analysis of such a linear, time-invariant system (Dorf and Bishop, 2008). Step inputs can also be used to estimate its steady-state characteristics (Hengstenberg, 1988; Goulard et al., 2015), and random sequences can be used to reveal non-linearities (Marmarelis and Marmarelis, 1978; Aptekar et al., 2014). However, these experiments can require a large number of individual experiments to reveal the response of the system over a range of inputs, or in the case of random noise sequences, a long experiment over which behavioural changes in response can occur. The provision of wild-caught insects can be limited and in some cases they do not survive for long after being transported to the laboratory, so a more efficient methodology was sought.

A recent experiment on gaze control in wasps (Viollet and Zeil, 2013) made use of a chirp stimulus—a sinusoidal waveform with a continuously changing frequency. The applicability of this stimulus to similar experiments on flies has not been tested, so results are presented here and compared to those obtained with constant-frequency sinusoids (CFSs). A longer, bandwidth-limited Gaussian-noise stimulus was
2. Behavioural paradigms for investigating gaze stabilisation

also tested to explore non-linear behaviour in the gaze control system and verify the
findings of Schwyn et al. (2011).

2.2 Materials and methods

2.2.1 Animal preparation

Female blowflies, *Calliphora vicina*, aged 5–13 days were collected from a captive
bred colony, subject to 12-hour light/dark cycles and maintained at 25°C. The ani-
imals were temporarily immobilised by cooling on ice and then manually positioned
to align a cardboard tether approximately perpendicular to the flat of the dorsal
prothorax, as viewed both frontally and laterally. Melted beeswax was used to rig-
idly join the tether and the thorax. Flies were allowed to recover for a few minutes
and then checked for unimpeded movement of the wings, approximately even stroke
amplitude of both wings, and a normal flight posture with the legs raised. White
acrylic paint was used to paint markers between the eyes in order to aid tracking of
the head in the subsequent video analysis.

2.2.2 Equipment

The cardboard tether holding the fly was secured to a step motor, controlled by one
of two micro-stepping drives (Astrosyn P808), with a resolution of either 3200 or
5000 steps per revolution. A switch allowed the motor to be controlled by either of
the two drivers, with one for faster stimuli requiring larger step sizes, and one for
slower stimuli. The signals used to drive the motor were output from Matlab (Math-
works, Inc.) at 100,000 samples/s, via two NI-6025E DAQ (National Instruments)
digital-analog converters. A false-horizon was created with a plastic hemisphere,
approximately 5 cm diameter, painted black, which was positioned underneath the
fly with the edge close to the eye equator. A larger diameter plastic dome which
encompassed the horizon was then lowered over the fly to form a white, translucent
dorsal hemisphere. Through this, diffuse illumination was provided by two white LED lamps with two ‘goose-neck’ light-guides each (Schott KL 1500). Luminance through the diffuser was measured to be 500 Cd/m². Additional filters could be inserted into the lamps to provide illumination in the red end of the visible spectrum only. Since the photoreceptors of the blowfly are insensitive to red light (Stark et al., 1979), this allowed for the reflex to be investigated without visual input, while providing sufficient illumination for the camera to operate. Frontal airflow was applied continuously to encourage flight.

A Fastcam SA3 (Photron USA, Inc.) high-speed camera was used with a 100 mm macro lens to record initial experiments. For experiments which required longer bouts of filming, a Phantom v211 (Vision Research, Inc.) was used with a Sigma 180 mm macro lens. Aperture sizes were adjusted between f/3.5 and f/5.6, depending on the use of the red filter. Framerates up to 1200 frames/s were chosen according to the velocity of the stimulus, ensuring at least 3 frames per degree of rotation.

Previous studies tested the head response at up to 15 Hz and a similar or higher range was sought in these experiments.

Figure 2.1: Setup of behavioural experiments. Left: Fly tethered to step motor with a false-horizon and diffuse illumination from above, mimicking skylight. Right: Conventions for the analysis of the head response, where relative head-roll, HR, is positive in the opposite direction to absolute head-roll and thorax-roll, HA and TR.
2.2.3 Conventions

Conventions for specifying roll angles of the head follow those used by Schwyn et al. (2011). The angle of the head as measured from the laboratory reference frame is referred to as the ‘absolute head-roll’ angle, HA. For the case of perfect stabilisation, HA = 0°. The output of the motor system during perfect stabilisation was defined as movement equal and in opposite direction to the thorax-roll. From the angle of thorax-roll, TR, the ‘relative head-roll’ angle, HR, was calculated:

\[ HR = TR - HA. \]

Note that the positive direction for HR is opposite to that of TR.

2.2.4 Video analysis

Video footage was analysed semi-automatically to extract the roll angle of the head and of the cardboard tether, which equals the roll angle of the thorax. Analysis was performed in LabView (National Instruments) using a template-matching method, as developed by Parsons (2008). The white markers on the head and tether increased contrast and provided good areas for templates to be defined (see Figure 3.1). The template-matching algorithm returned a score based on the confidence of the match for each frame. Where scores were low for particular frames the roll angles were interpolated using the values from neighbouring frames.

2.2.5 Conditions

C1 The intact condition refers to experiments performed on the fly in its *unscathed state*. Only active, flying flies were picked and the wings were checked for damage.

C2 *Ocelli occluded* refers to experiments performed on the same flies after painting over the dorsal ocelli.

C3 The *dark* condition probes the input of the halteres and mechanosensors alone, without visual input. A curtain was used to exclude all light from
outside of the experimental setup. Only red light for the cameras was present, which does not stimulate the fly visual system.

C4 *No halteres* refers to experiments performed on the same flies after cutting the halteres at their base, to probe the performance of compound eye input alone with only minor sensory modalities such as the antennae and neck mechanoreceptors.

C5 *No halteres, dark* refers to experiments performed on the same flies with neither visual input nor haltere input.

### 2.3 Chirp stimulus

#### 2.3.1 Design and implementation

The main motivation for using a swept-frequency chirp stimulus was to reduce the time that the fly spent in the behavioural setup, by investigating stabilisation responses at a range of frequencies within a single experiment. The range of frequencies tested in a single chirp was chosen to focus on the higher velocities, above 10 Hz, where the limits of performance of the gaze stabilisation system have been observed in previous work (Hengstenberg, 1984; Schwyn et al., 2011). A linear sweep of frequencies was chosen in order to simplify the analysis and to avoid the possible confounding effects of adaptation of the animal’s response at a particular frequency. An amplitude of ±30° was chosen to match previous experiments, and a frequency range increasing from 0–20 Hz in 5 seconds.
The stimulus time-series was defined as follows:

\[ x(t) = \sin \left( 2\pi f_0 t + \pi rt^2 \right), \]

where \( f_0 \) is the initial frequency, \( t \) is the time vector, and \( r \) is the chirp rate—the rate of change in frequency defined over the length of time, \( T \), as:

\[ r = \frac{f_{\text{max}} - f_0}{T} \]

In order to examine the effect of accelerating and decelerating frequency, the stimulus was run in reverse immediately upon reaching the maximum frequency. Potential effects from sensory response delay, or lag, and hysteresis in the response of the neck motor system could cause non-linear effects as the input and outputs grow out of phase by greater than one cycle. To identify these effects, the progression of each individual response was analysed cycle-by-cycle. Checking over the increasing and decreasing frequency ranges also ensured that an asymmetrical response profile could be detected.

**Figure 2.2**: Chirp stimulus spectrogram. Top: Example time-series of stimulus output from motor, analysed through template-matching on video. Bottom: Signal power over the time-series.
A spectrogram showing the power of the stimulus time-series is illustrated in Figure 2.2, showing the Fourier spectra of one-second sequences of the stimulus, each overlapping by 90%. This window length was specified to ensure a sampling rate of twice the period of the lowest frequency component to be analysed—around 0.5 Hz.

For a constant-frequency sine (CFS) stimulus, the signal power is concentrated in a narrow frequency band. Since the chirp stimulus had a continuously changing frequency, the signal power was spread more widely, as shown in the spectrogram in Figure 2.2. This resulted in a lower signal-to-noise ratio at any particular frequency, so a higher number of trials were tested than in previous investigations making use of CFSs. Seven trials were performed for each fly. Despite this increase, and the long stimulus length, the total recording time required was not longer than for individual CFS experiments, and the protocol was simplified from running ten or more individual protocols for each condition to just one.

2.3.2 Results

Blowflies responded to the chirp roll stimulus with compensatory head movements, in an attempt to stabilise the head. Mean traces of the relative head-roll at different sections throughout the stimulus are presented in the top row of Figure 2.3. The rightmost column shows the section with the highest frequencies tested, where the response amplitude had decreased compared to that of the lower frequency section (centre column). In each section, the response lagged behind the input by less than a quarter of a cycle. For C2 (second row), very little effect is discernible from the occlusion of the ocelli. The third row shows the response in the simulated dark condition with no visual input (C3) and, interestingly, only a slight reduction in amplitude is visible between the two frequency ranges.
2. Behavioural paradigms for investigating gaze stabilisation

2.3.3 Slip speed increases in the absence of haltere input

For the two conditions tested after removing the halteres in Figure 2.3, shown in orange, the mean responses were more prominently reduced in amplitude, and lagged by at least a quarter of the stimulus cycle. At the highest frequencies shown, the phase delay was over 180° for both conditions C4 and C5, representing movement of the head which added to the roll induced by the stimulus. To quantify the consequence of this increased rotation on the effectiveness of the gaze stabilisation reflex, the retinal slip speed was calculated, defined as the relative angular velocity between the head and the environment. Perfect compensation would maintain the retinal slip speed at zero, although reducing it to around 200°/s should stabilise the head sufficiently to avoid motion blur (van Hateren, 1992).
Figure 2.4 shows the probability density function of the retinal slip speeds for the different conditions tested with the chirp stimulus, and for the thorax-roll input, TR, which is interpreted as the retinal slip speed experienced if the head were fixed to the thorax. For the conditions in which the halteres were intact, the slip speed distributions showed a high degree of overlap. Compared with the distribution of the input, these were shifted towards lower angular velocities, as expected with stabilisation of the head. For both conditions with no halteres, the distributions were close to that of the input. With angular velocities above 3000°/s the distributions were shifted towards higher velocities, showing that without the input of the halteres the gaze stabilisation system increased the retinal slip speed.

![Distribution of slip speeds](image)

**Figure 2.4:** Distribution of slip speeds in response to the chirp stimulus. The normalised probability of occurrence of each slip speed velocity, calculated from the mean response trace for each condition. Mean of N = 8 flies, n = 7 trials, error bars show ± standard error.

### 2.3.4 Gain and phase

In order to analyse the performance at a given frequency of the chirp stimulus and compare the results with those obtained using constant-frequency sine stimuli, the frequency response was plotted in terms of the gain and phase of head movements (Figure 2.5). The time series of the response was divided into short sequences, each overlapping by 90%, as before, and the Fourier spectrum was found for each. This
process was necessary since the non-stationarity of the chirp signal made it unsuitable for analysis via a single Fourier transform. The gain and phase of the relative head-roll were estimated for the peak frequency component in the 74 overlapping time sequences of the mean response data. This is presented for the first 5 s, in which the frequency of the stimulus increased, and for the last 5 s, during which it decreased.

![Figure 2.5](image)

**Figure 2.5:** Relative head-roll gain and phase in response to the chirp stimulus. Mean of $N = 8$ flies, $n = 7$ trials, error bars show ± standard error.

Examining the gain of the response during the increasing chirp rate, the results can be seen to fit with those observed in Figure 2.3. The general trend of the gain for the conditions with haltere input (blue) was to decrease as frequency increased, showing a decrease in performance previously found using CFS stimuli (Hengstenberg, 1993). Here, the very slight difference between conditions C1 and C2, is apparent around the peak at 10 Hz, although throughout the rest of the range, occluding the ocelli appeared to have no impact on the gain. With no visual input, the gain for C3 was reduced by approximately 0.1 units. The phase was relatively flat across the range of frequencies, and well above $-90^\circ$. The phase for
C3, in the dark, was slightly decreased compared to C2, in the light, at 12 Hz and above. This seems to indicate that the loss of compound eye input was not detrimental at high frequencies, which fits with previous results shown in Figure 1.3. Head responses were made slightly faster with the halteres alone, albeit with lower gain.

The gain was much reduced by removing the halteres, shown in C4, decreasing to almost zero at around 12 Hz. This was exacerbated by removing visual input, in C5, and the response gain was shifted downwards by a similar, or greater, amount than observed for removal of visual input while the halteres were present, between C2 and C3. The phase delay for both conditions without halteres was significantly increased compared to those with halteres, and reached $-180^\circ$ at around 15 Hz. At that point, the gain was slightly increased, however this should not be misinterpreted as an increase in performance: the phase continued to decrease as the frequency increased, and the slip speeds were increased, as shown in Figure 2.4.

For the subsequent response to the decreasing chirp rate, the same trends were observed and the results from the two were qualitatively similar. Between 2–15 Hz, however, a leftwards shift of around 3 Hz is noticeable for all gain curves. The phase does not appear to have been affected in a similar manner. The chirp rate decreased during this sequence, so the lower frequencies were towards the end of the trial. The effective decrease in gain at low frequencies could therefore have been due to a slight adaptation to the response.

Between 2–5 Hz, the error of the phase estimate was considerably larger than at higher frequencies. This was due simply to the lower number of cycles of these frequencies contained within the stimulus—the estimate was subject to greater noise. The lack of data for frequencies below this range is a result of the windowing in the analysis; in the first and last time-windows used to obtain the short-sequence
Fourier spectra, the peak frequency component was around 2 Hz, so the mean gain and phase estimates for these sequences were assigned to 2 Hz.

2.3.5 Comparison with constant-frequency sinusoidal stimuli

The gain and phase plots allowed for a direct comparison with previous work, which tested conditions comparable to C2 and C4, using constant-frequency sinusoidal stimuli (Hengstenberg, 1988; Schwyn et al., 2011). The mean of the increasing and decreasing chirp sections of the stimulus were used for this comparison.

The results were qualitatively similar, with slight quantitative differences. Only the phases for C2 appear to be the same in both sets of results. The gains for both C2 and C4 appeared to be lower in the chirp response, and the effect of removing the haltere input was much more pronounced. The same was true for the effect on the phase, and in fact the response obtained from the CFSs did not reach $-180^\circ$ phase within the frequency range tested. In this respect, the range of the chirp stimulus was beneficial, however the quantitative differences at lower frequencies suggests
2. Behavioural paradigms for investigating gaze stabilisation

that this could be an effect of the chirp stimulus. Another advantage of the chirp stimulus was the continuous sampling of the frequency range. Between 1–6 Hz on the CFS plot, the trend of the gain with frequency is potentially misleading when viewed in isolation, due to the coarse frequency sampling. The chirp plots overcome this with a more detailed estimate.

2.3.6 Discussion

The chirp stimulus was determined to be suitable for stimulating compensatory head-roll reflexes of the blowfly, and allowed for the contribution of the different sensory inputs to be teased apart. The frequency-shift of the gain, seen between the increasing and decreasing chirp sequences, is interesting and could potentially indicate a problem with the methodology, such as an effect of sustained acceleration, or it could indicate an adaptation of the response. The difference was small, though, when compared to the difference between using the chirp and the CFS stimuli. The gain and phase values were not easily comparable across the methods. This suggests that other parameters, such as retinal slip speed, could also not be quantitatively comparable between the different experiments. However, a comparison for the hoverfly in Section 3.2.5 presents a much closer match. Here, the relationships between gain, phase and frequency of the head-roll response appear to be only qualitatively comparable.

The analysis was straightforward with the linear chirp tested here. There was a lack of coverage of frequencies below 5 Hz, which could be improved with a logarithmic chirp rate, and in the work by Schwyn et al., frequencies were tested down to 0.01 Hz. However, the period of such low frequencies would add greatly to the length of the stimulus. These could be run individually with constant frequency sinusoids and combined into the same dataset. For the blowfly, the response gain is known to be close to unity at frequencies below 1 Hz (Schwyn et al., 2011), so the incompatibility of gain obtained with the two methods would not be of concern.
Moreover, it is the high frequency components at which the limit of performance is tested, which is the focus of this investigation.

### 2.4 Random-noise stimulus

#### 2.4.1 Design and implementation

A Gaussian white-noise stimulus is an alternative method for probing biological control systems, and is well-suited to systems identification of sensorimotor reflexes, where non-linear behaviour may be expected (Marmarelis and Marmarelis, 1978). Although previously shown to be largely linear, the gaze control system of the blow-fly is likely to show some degree of non-linear behaviour, and for other species the same linearity cannot be assumed. Using the same setup and motor as used for the experiments with the chirp stimulus, a white-noise approach was implemented to assess whether a response of the head could be observed and whether it would prove useful for further application to other species.

![Bandwidth-limited noise stimulus](image)

**Figure 2.7:** Bandwidth-limited noise stimulus. Top: Time-series of roll angle over the duration of the experiment. Bottom: Normalised probability density function of the roll angle of the stimulus, and the angular velocity applied to the thorax as a result.
2. Behavioural paradigms for investigating gaze stabilisation

The time-series of the stimulus was specified using a fixed time-interval, chosen to be long enough to observe the response mediated by the slowest sensory modality, the compound eyes: on the order of 30 ms. Time-intervals of 120 ms were therefore used. A rotation amplitude was randomly chosen from a Gaussian distribution and assigned to each time interval, so that the stimulus contained short sequences of constant velocity, in alternating directions. The angular velocities in the stimulus then also followed a Gaussian distribution, shown in Figure 2.7. The length of the time-series was 50 s, the longest possible with the Fastcam SA3 camera capturing images at 500 frames/s. The last rotation of the stimulus was manually set to return the motor to its starting position at 0°. Once specified, the same time-series was used in each experiment.

The bandwidth of the stimulus was constrained by the step-motor drivers, and what could be physically realisable. The accuracy of the stimulus representation output by the motor was affected by the resolution of the driver. The choice of driver was a trade-off between a higher bandwidth with coarse sampling, and more accurate sampling with a lower velocity limit. Preliminary tests revealed that the coarsely sampled input signal caused errors in the motor output at reversal points where acceleration was high. The output signal often became unpredictable, so this option was dismissed. This was a significant limitation, and the input signal did not represent a ‘white’ noise stimulus, as such. The fly’s response to this type of stimulus was highly interesting nonetheless. The peak angular velocity of the thorax-roll was limited to just over 600°/s, theoretically high enough for the fly to experience significant blurring if uncompensated (van Hateren, 1992).

2.4.2 Results

The experimental conditions for testing different sensory modalities were the same as for the chirp experiments, with the exception that the ocelli were occluded in all cases, so C1 was excluded. 8 flies were tested and were able to fly for the duration
of the 50 s stimulus under all conditions except one, C4 (no halteres, dark). Only a single fly performed in this condition, so the data are not presented here.

![Graphs showing mean head-roll responses for different conditions](image)

**Figure 2.8:** Mean head-roll response to random-noise stimulus. Extracts of the mean response are shown for each condition, left: at the start of the experiment, and right: midway through. (HR is shown, so traces represent movement in the opposite direction to the thorax). N = 8 flies, n = 10 trials, error bars show ± standard error.

Flies responded to the roll rotations of the thorax as in the experiments described in Section 2.3.2, by making compensatory head movements in the opposite direction. Figure 2.8 shows the mean head-roll responses for two separate sequences of the stimulus. The effect of removing the halteres was clear from the mean response traces: the gain was decreased, the peaks lagged further behind those of the stimulus, and the sharp deceleration and reversal seen for C2 was ‘smoothed’ out, akin to a low-pass filtering operation on the time-series. This supports the current view that the halteres are responsible for the high frequency components of the compensatory head movements (Hengstenberg, 1988; Schwyn et al., 2011).
To investigate the gain for each of the individual 120 ms velocity intervals of the experiment, the thorax-roll signal was analysed without assuming a priori knowledge of the stimulus design, and the head-roll amplitude was found within each interval. The Hilbert transform of the signal was applied to find the reversal points of the stimulus trace, and the amplitude of relative head movements then found for each interval between these points. The gain of these head movements—the ratio of the relative head-roll to the thorax-roll amplitude—is shown for each velocity within the stimulus in Figure 2.9. Between 0–50°/s, high variation was seen for C3 and C4, with responses spread between 0 and 1. At higher velocities, the gain was flat in all conditions. The decrease between successive conditions, observed previously for the chirp stimulus, was also apparent from the fitted lines, with a constant gain of around 0.5 and 0.45 for C2 and C3, respectively. The gain for C4 follows a more obvious decrease with velocity, with a gain of around 0.3 at the highest speeds, which qualitatively compares with the results from Schwyn et al. (2011).

Figure 2.9: Gain and slip speeds in response to noise stimulus. Left: Gain of head-roll response for each 120 ms section of the stimulus. Lines show least squares fit of a 1\textsuperscript{st} order polynomial for each condition. Right: Normalised probability density of retinal slip speeds obtained using the absolute head-roll angle, HA. Mean of N = 8 flies, n = 10 trials, error bars show ± standard error.

Retinal slip speeds were calculated as for the chirp experiments. With the halteres, a slip speed below 200°/s was maintained throughout almost the entire stimulus
(both blue curves). Without halteres, the slip speed distribution was still shifted towards lower velocities by the gaze stabilisation system, using predominantly visual information through the compound eye pathway (the ocelli were occluded here). However, the stimulus did not reach high enough velocities to show the crossover of the TR and C4 curves seen in the same plot of slip speeds for the chirp experiments (Figure 2.4). The power spectra of the stimulus and mean responses were obtained from the Fourier transforms of the time-series (not shown). The gain and phase information calculated from these were similarly flat, as in Figure 2.9, and offered little insight.

**2.4.3 Discussion**

The noise stimulus, in the form implemented here, was of limited use for investigating gaze stabilisation performance in the blowfly. The maximum angular velocities experienced by the fly were less than one fourth of those for the chirp stimulus, and the stimulus also lacked the extended periods of slow, gradual movements necessary to examine responses to low velocities. As such, the analysis of these results was not taken further, since it was considered unlikely to yield more information than had been obtained from the chirp and CFS stimuli.

Encouragingly, blowflies could be subjected to experiments of 50 seconds under all but the most demanding conditions, and continued to fly and respond throughout. An alternative design for the stimulus which may be better suited to investigating gaze behaviour would replace the fixed time-step length with a fixed rotation amplitude. A random distribution of angular velocities would be used, each applied for the time necessary to rotate to reach the specified angle. In this manner, the fly’s response to low velocities could be analysed over a longer period. With a bandwidth one order of magnitude higher, the results from a quasi-white noise stimulus could be highly informative.
2. Behavioural paradigms for investigating gaze stabilisation

2.5 Concluding remarks

In this chapter, two stimulus paradigms were tested for investigating the gaze stabilisation of the blowfly, *Calliphora vicina*, and compared with a third. The chirp stimulus was found to show a variety of phenomena observed in previous experiments which used constant-frequency sinusoidal (CFS) input stimuli. These observations were qualitatively similar, but quantitatively quite different; removing the halteres, for example, caused a much greater increase in the response delay to the chirp stimulus than for the CFS stimuli. This particular effect could be due to the continuously changing frequency: there is little time for the fly to adapt to a particular frequency in the chirp stimulus. The under-representation of low frequencies in the stimulus is also problematic for the investigation of previously untested species, since they may use different strategies for slow and fast disturbances, which could be missed in their response, as will be shown for hoverflies in Section 3.2. The major advantage of the chirp stimulus is its practicality—a single stimulus which covers a wide range of input frequencies saves time and effort over testing a subset of frequencies individually. This is offset somewhat by the need for a greater number of trial repeats, but the savings are nevertheless substantial. The task of video analysis is also simplified, since there are fewer videos.

On the other hand, the noise stimulus proved to be less useful. The range of velocities in the stimulus distribution was limited by the performance of the step-motor driver used here, and this in turn limited its use for probing behaviour. A motor driver with an adaptable step resolution could solve this issue, but was not investigated here. For further investigations, a modification would be made to the implementation of the stimulus: using a random distribution of rotation durations at constant velocity and fixed amplitude, rather than a random distribution of amplitudes with a fixed rotation duration. While this may be interesting to investigate
2. Behavioural paradigms for investigating gaze stabilisation

in the blowfly, a method using sinusoidal stimuli—linear chirp or constant frequency—was preferred for the comparison of behaviour across species. This maintains the main characteristic rotational movement used in numerous previous studies, in order to make direct comparisons. For the proceeding experiments a mixture of chirp and CFS stimuli are used. Further comparison in other species in the next chapter clarifies the suitability of each.
3. Head-roll stabilisation in three Dipteran families

This chapter presents a comparison of the characteristics and performance of roll gaze stabilisation behaviour of flies from different Dipteran families. Species were chosen with dissimilar flight behaviours, or with particular features that contrast with the blowfly. The distinguishing characteristics of each are summarised in Table 1.1.

Section 3.1 presents the results of gaze stabilisation experiments on the horsefly, *Tabanus bromius*, which resembled the behavioural results for the blowfly, presented in Chapter 2. Section 3.2 details a different form of stabilisation observed in 3 species of hoverfly. It is suggested that the inertia of the head plays an important role in providing stabilisation at high angular velocities. Finally, Section 3.3 presents findings from experiments on the robberfly, *Philonicus albiceps*, which highlight the significance of the ocelli in this insect—further investigated in Chapter 4.

3.1 Horsefly

3.1.1 Introduction

Horseflies (Tabanidae) are infamous for the bites they inflict as they suck the blood of mammals, including humans. Females require this blood meal for the development of their eggs (Marshall, 2012) and on sunny days during July–August in Britain they can be found seeking out and feeding on cattle and horses (Lehane, 2005). The sensorimotor reflexes of Tabanidae are not commonly studied, although adaptations of the visual system which allow them to detect polarised light in their ventral visual field—which aid the location of prey and bodies of water for reproduction (Blaho et al., 2012)—distinguish them from other Dipteran flies. Most species also lack the simple eyes known as ocelli which contribute to gaze and flight stabilisation in other
flying insects (Stubbs and Drake, 2001)(see Chapter 4 for further details). In the light of this reduction in visual senses, horseflies make an interesting comparison for this study. Their flight behaviour may be specialised for approaching targets quickly and landing undetected.

3.1.2 Materials and methods

Female *Tabanus bromius*, were caught in fields near Amersham, Buckinghamshire, cooled with ice, and transported to the laboratory where they were kept with sugar and water. Four flies were tethered and chirp stimuli were applied to the thorax, using the methods described in Chapter 2. Constant frequency sinusoidal (CFS) stimuli were also applied at 3, 6 and 10 Hz for 10 seconds per trial. 1 Hz stimuli were applied for 20 seconds and 0.1 Hz stimuli were applied for 100 seconds, per trial.

![Figure 3.1: Image of the horsefly, *Tabanus bromius*. (Courtesy of Steven Falk)](image)

Right: Video frame from roll behaviour experiment. The red box indicates the matched template from which head angle, HA, was estimated. The head is shown to be level with the horizon, indicated by the white dashed line (HA = 0°), while the template matched to the painted pattern on the tether indicates a thorax-roll, TR, of approximately 30°.

3.1.3 Results

Preliminary responses to chirp stimuli revealed that horseflies make compensatory head movements in much the same manner as blowflies: as the thorax was rotated
away from the default orientation, compensatory movements of the head in the opposite direction were made to maintain a level gaze, as shown in Figure 3.1. The mean absolute head response (HA) of four intact flies to the chirp stimulus is shown in Figure 3.5. The roll angle of the head was reduced relative to the thorax angle, but not abolished completely, and effectiveness decreased as frequency increased.

Since the analysis of the chirp stimulus in the frequency domain, presented in Section 2.3.5, indicated discrepancies with the results obtained from CFS experiments, as well as poor coverage of low frequency responses, the results from the CFS experiments on horseflies are presented here. Figure 3.2 shows the frequency response obtained for the horsefly head.

![Frequency response: horsefly](image)

**Figure 3.2:** Frequency response of the horsefly head to a thorax-roll rotation. Mean head-roll gain (top) and phase (bottom), for N = 4 horseflies, n = 2 trials, error bars show ± standard error. The phase plot shows a longer delay for conditions tested without halteres, shown in red.

For the intact fly, the maximum response gain was found for a body oscillation frequency of 1 Hz. The gain of 0.85 indicates the ratio of the output, head-roll (HR), to the input, thorax-roll (TR), and a comparable gain to the blowfly response at 1 Hz (approximately 0.75). The gain dropped at both lower and higher frequencies,
to about 0.6 at 0.1 Hz, and 0.5 at 10 Hz. The phase delay of the compensatory head-roll response, relative to the forced thorax rotation, reflects the processing time of the sensory input and the time taken for the neck motor system to rotate the head relative to the body. With all sensory systems intact, the phase delay was consistently close to 0° over the entire frequency range tested, demonstrating a fast response that incurred very little response delay (Figure 3.2, bottom). In the dark—when no visual input was available to the flies—the gain was decreased over the entire frequency range compared to the control condition where the setup was illuminated.

The phase delay under the dark condition was virtually the same as for the illuminated conditions, suggesting an important role for the halteres, or other mechanosensory mechanisms; mechanosensors appear to maintain the fast response of the system, but with head movements of lower amplitude. It is likely that for the horse-fly, as postulated for blowflies, the halteres provide a rapid feed-forward estimate of thorax-roll to the neck motor system, which then receives feed-back from the visual system to correct the remaining error in head angle (Schwyn et al., 2011). In the dark condition, this visual feed-back was removed, resulting in a lower, less-effective overall gain in compensatory movements Figure 3.2.

This interpretation is further supported by the data presented from flies with no halteres (Figure 3.2, bottom, red traces): the phase delay was much greater at high frequencies, passing −90° in both the light and the dark conditions, with some flies generating head-roll almost in anti-phase with the thorax rotation. The gains were significantly lower for both haltere-less conditions and, again, animals flying in the dark made head movements with lower gains than those flying in the illuminated setup. The peak response was found to be at 1 Hz, as before, with comparatively high gains.
3. Head-roll stabilisation in three Dipteran families

At 1 Hz, the response was not greatly affected by the removal of the halteres, and appears to be dominated by the contribution of the visual system. However, in the dark, the gain of the response for flies without halteres was only reduced to 0.7, demonstrating a surprisingly effective stabilisation compared to the gains at other frequencies, despite a lack of two supposedly critical sensory systems. It is also interesting to note that at the lowest frequency tested, 0.1 Hz, flies demonstrated the same response gain under all conditions—also approximately 0.7. This raises the question of whether low velocity rotations are detected by another mechanosensory modality, which will be discussed in Section 3.1.5.

3.1.4 Significance of haltere input

Recent work on the gaze stabilisation system in blowflies by Heras et al. (in preparation) has further demonstrated the importance of the halteres. A similar observation was made for the horseflies studied here, indicating a commonality in control systems design. Figure 3.3 shows the mean distribution of retinal slip speeds experienced by the horsefly (top) and the blowfly (bottom) at different stimulus frequencies. The slip speed corresponds to the angular velocity of the head relative to the environment. At 1 Hz, the slip speeds experienced by both flies were distributed within a range of 0–100°/s. The peak velocity of the thorax (vertical grey line) was just below 200°/s, so the movement of the head relative to the thorax effectively maintained lower slip speeds than the hypothetical situation in which no attempt to stabilise is made and the head is locked with the rotation of the thorax. For the condition with halteres (blue, both flies), the peak and median of the slip speed distributions are shifted to the left of the peak thorax velocity. On the other hand, conditions without halteres (pink, orange) stabilised less effectively, as indicated by the rightwards shift of the distributions.

Figure 3.4 shows the median of each slip speed distribution across the frequencies tested. The grey lines indicate the median of the thorax-roll (TR) velocity, which
increased linearly with frequency. For the blowfly (right), median slip speed remained below the median TR velocity at all frequencies for the intact condition. For the condition tested without halteres, the median slip speed distribution at 10 Hz was above the median TR velocity—meaning that on average the slip speed was increased by making movements of the head relative to the body. This was also the case at 15 Hz.

![Figure 3.3](image)

**Figure 3.3:** Retinal slip speed distribution during CFS thorax-roll experiments. Mean probability density function (PDF) of the slip speeds experienced for horsefly (top), N = 4, and blowfly (bottom), N = 5, error bars show ± standard error.

![Figure 3.4](image)

**Figure 3.4:** Median slip speed during CFS thorax-roll experiments. Left: horseflies, Right: blowflies. Median of the slip speed distributions shown in Figure 3.3. Error bars show ± standard error.
On the left of Figure 3.4, the same trend of increasing median of the slip speed distribution, and eventual crossover, can be seen for the horseflies. At 10 Hz, the median slip speed without halteres was almost identical to the median TR velocity, and at 15 Hz the slip speed was higher, as in the blowfly. These plots show a response of the gaze stabilisation system in both flies with a phase which has passed $-180^\circ$ at high frequencies, in the absence of the rapid feed-forward input from the halteres. Compound eye-mediated head responses are clearly not fast enough to cope with high velocity rotations of the stimulus and as a result, delayed compensatory movements of the head can result in an increase in slip speed, rather than a decrease.

This serves as a functional interpretation of the physical meaning of the frequency response plot in Figure 3.2: without halteres, the low gain and approximately $-180^\circ$ phase of head movements in response to 10 Hz roll rotations corresponds to an increase in the median slip speed distribution, compared to a situation in which the fly could lock its head to its thorax.

3.1.5 Discussion

This is believed to be the first investigation of stabilising head movements in horseflies. The species of horsefly studied here was found to be highly amenable to tethered flight experiments; the animals flew continuously more readily than hoverflies and robberflies, and showed stabilisation of the head which resembled that of blowflies. The apparent lack of functional ocelli certainly did not appear to prevent the horseflies from making fast head movements; thorax-roll of up to $1000^\circ$/s (3 Hz) was well compensated. At higher speeds, the average gain of the horsefly’s gaze stabilisation system was comparatively lower than the blowfly’s (approximately 0.5 vs 0.75, respectively), and the delay slightly longer. The contribution of the ocelli in other species is considered in detail in Chapter 4, however it is not clear that their input alone could account for the difference observed between horsefly and blowfly
responses at 6 and 10 Hz. A number of other factors could be at play, including differences in physical size and mass of the head, sensory processing delays, and the biomechanical limitations of the neck motor system.

Free-flight behaviour of horseflies has not been well-documented, so the requirements for gaze stabilisation in the context of their ecology can only be speculated on. Females of the species studied in this investigation were frequently caught while resting on fence posts or similar raised objects surrounded by fields, often with cattle grazing. Flight was observed as either in a straight line across fields or as agile manoeuvres around targets or landing sites. Horseflies were not seen making saccadic, ‘search’-type flight, which involves high angular velocity roll turns in blowflies and requires equally fast stabilising head movements (Schilstra and van Hateren, 1999).

When interpreting the results of this work it is important to keep in mind that the reflexive head movements were measured in response to external perturbation. During voluntary turns involving thorax-roll, head movements executed to stabilise vision may not be limited to the performance measured here. A forward signal, or efference copy, from areas of the nervous system issuing the motor command to turn (review: Webb, 2004; Kim et al., 2015), could initiate movements of the head without the delay incurred by sensory pathways which is inherently measured in the responses here. For that reason, failure to effectively compensate for thorax-roll at high angular velocities in these experiments, particularly by robberflies, does not rule out the possibility of adequate compensation during voluntary turns of equal velocity during flight.

When experiments were conducted under dark conditions, surprisingly little impact on the head response was observed, even after the flies’ halteres were removed. How does the horsefly’s gaze stabilisation reflex operate without the estimates of head and thorax angle provided by the compound eyes and halteres? Additional
visual inputs as a result of the red light used for filming were discarded as none of the photoreceptor types of *Tabanus bromius* is known to be strongly sensitive to red light (Belušič, 2015, pers. comm.), so the assumption of no visual input would appear to be valid. Of the other sensory modalities which are known to influence roll gaze stabilisation in the blowfly, the wing campaniform sensilla could account for the detection of thorax-roll in the absence of the halteres. Situated at the base of the wing, they sense wing loading and deformation, and could potentially detect an imbalance due to thorax-roll (Hengstenberg, 1988).

Passive, mechanical stabilisation of the head, which would rely less heavily on sensory input in relation to hoverflies is discussed in the next section, and could offer an alternative explanation. Intriguingly, some species of horsefly are known to hover and make agile manoeuvres in flight, much like hoverflies (Wilkerson and Butler, 1984).
3. Head-roll stabilisation in three Dipteran families

3.2 Hoverfly

3.2.1 Introduction

A far larger body of work exists on the study of hoverfly neuroethology than horseflies or robberflies. Studies of hovering using high-speed videography have helped describe their behaviour and the strategies employed for visual control of flight and target tracking (Collett, 1980). Collett and Land (1975) reported that the small hoverfly *Syritta pipiens* (which has a similar body morphology to *Episyrphus balteatus*, studied here) does not make compensatory head movements in flight like the blowfly, and using magnetic search coils mounted on the larger *Eristalis tenax*, Schilstra (1999) showed that the head is not stabilised during roll turns, and instead follows the movement of the body, after a short delay. Strategies for stabilising gaze by rotating the entire body have been described, however, which perform a similar function to the ‘saccade and fixate’ and smooth pursuit movements of vertebrate eyes (Collett and Land, 1975).

In terms of neuronal architecture, 3 species of hoverfly have been shown to have one more HS-cell than the blowfly, *Calliphora*, in the lobula plate, and their response to motion is similar (Buschbeck and Strausfeld, 1997; Nordström et al., 2008). There is evidence that homologous neurons are slightly faster in *Eristalis*, with a larger receptive field (Geurten et al., 2012). The number of VS cells varies and some species may possess twice the number that *Calliphora* does (Buschbeck and Strausfeld, 1997). Their axons and main vertical branches have similar diameters, suggesting the importance of detecting vertical motion in any region of the eye; Collett and Land (1975) showed that target fixation and target oriented hovering can be mediated equally well by either the frontal or lateral regions. In addition to being capable of almost stationary hovering, hoverflies may translate along almost all axes relative to their bodies, and do not necessarily fly forwards (Collett and Land, 1975; Geurten et al., 2010). This is significant in that the focus of expansion of optic flow processed
by the VS cells—from which rotational self-motion is likely to be extracted—may not be centred in the frontal region (Geurten et al., 2012).

3.2.2 Initial behavioural observations

Preliminary behavioural experiments on wild female *Eristalis tenax* (Figure 3.7) found that compensatory head movements were made in response to forced thorax-roll, as previously described for the blowfly and horsefly, with additional periods in which the head was noticed to rotate more freely. During these periods, small, unsteady yaw and pitch oscillations of approximately 1–2° were observed, which gave the appearance of the head ‘wobbling’ around the neck joint. When high frequency (>10 Hz) thorax-roll oscillations were applied, the head appeared to be stabilised by the inertia of the head—as the thorax rolled sinusoidally, the head exhibited apparently smaller passive oscillations which did not appear to be under active control, and the rotation of the thorax did not appear to be transmitted through the neck motor system to the head.

3.2.3 Chirp response, *Episyrphus balteatus*

To investigate this further, and to determine whether the observed phenomenon was specific to *Eristalis tenax*, chirp stimuli were applied to another species of hoverfly, the smaller, narrower-bodied *Episyrphus balteatus*. Figure 3.5 shows the mean response of eight female hoverflies to the chirp stimulus (top), along with the mean response of the four female horseflies (bottom) tested in Section 3.1, for comparison. These plots present the absolute head-roll angle, HA, whereby perfect stabilisation of the head would be a constant amplitude of 0°.
3. Head-roll stabilisation in three Dipteran families

The mean head response trace of the hoverfly shows multiple stages: at the start of the stimulus, during low frequency rotations of the thorax, the head showed almost no relative movement and remained in-phase with the thorax rotation; at 1.5 seconds, when the stimulus frequency was around 6 Hz, the fly’s head began to move relative to the thorax, and the amplitude of head oscillations decreased, relative to the thorax; up to around 3.5 seconds (14 Hz), the oscillations of the head continued to decrease in amplitude as the frequency of the stimulus increased; from 3.5 seconds onwards the head oscillated around 0°, on average, but with some variability, seen as an offset of the trace; this continued until the reverse sequence of events occurred from 5–10 seconds. The amplitude of oscillations during the middle sequence was between 10–15°, corresponding to a gain of around 0.5–0.65. In comparison, the mean response trace of the horsefly head shows a lower roll amplitude than the thorax from the start of the stimulus, demonstrating compensation of the head angle, which decreased in effectiveness as frequency increased. At the midpoint of the experiment, where the frequency was highest (20 Hz), the amplitude of the head-roll was close to the amplitude of the thorax-roll, indicating a gain close to 0.

**Figure 3.5**: Mean chirp response traces. The plots show the mean absolute head-roll angle, HA, for the hoverfly, *Episyrphus balteatus*, (top) (N = 8), and the horsefly, *Tabanus bromius* (bottom) (N = 4). The thorax-roll, TR, is shown in grey.
This comparison illustrates the proposed stabilisation employed by *Episyrphus*, involving two states: a head-fixed state, in which the head appears to be locked with the thorax; and a loose-head state, as described in the previous section, in which high frequency rotations of the thorax are stabilised by the inertia of the head.

### 3.2.4 Further behavioural observations

During behavioural experiments, hoverflies were observed to make large amplitude roll movements of the head while in the putative loose-head state. The rate of occurrence of these extreme head movements was less than once per trial, and they did not appear to be correlated with the thorax-roll stimulus.

![Hoverfly head movements in free- and tethered-flight. Left: *Episyrphus balteatus* executes close to 180° thorax-roll within 40 ms (images proceed from left to right). The orientation of the head can be deduced from the position of the bright region between the two eyes in the ventral half of the head. Right: video frame from roll behaviour experiment on *Episyrphus*. The frame shows the head inverted, at a roll angle greater than 180°.](image)

The motion of the head disclosed an initial impulse applied by the neck to rotate the head, with its momentum proceeding to rotate it further with very little resistance. At 180–270°, the head would appear to hit a physical limit to rotation and rebound slightly, shown in Figure 3.6. The head could remain inverted as such for a few seconds before being corrected, or the rebound could carry the head back to its default orientation. This behaviour was not suspected to be an effort to stabilise the head, and more likely to be an effort to escape the tether. However, it demonstrates the flexibility of the neck of *Episyrphus*. The head was seen to move normally after these turns, which apparently did not damage the neck muscle system or, it is
assumed, the neuropil of the neck connective. The flexibility of the head, and the apparent low torsional stiffness, are discussed further in Section 3.2.8, in relation to the proposed inertial stabilisation of hoverflies, and similarities with the inertial stabilisation seen in dragonflies.

3.2.5 Frequency response, *Eristalinus aeneus*

To further understand the methods of stabilising head-roll employed by hoverflies, a third species was investigated. A round-bodied hoverfly, resembling a smaller *Eristalis* species, was obtained from captive-bred laboratory stock (Bioflytech, Spain).

![Figure 3.7: Photograph of male hoverflies, *Eristalis tenax* (left) and the smaller *Eristalinus aeneus* (middle). (Courtesy of Steven Falk). Right: Video frame of roll behaviour experiment on *Eristalinus aeneus*. The image shows a well-stabilised head at 25 Hz.](image)

Chirp and CFS stimuli were used to characterise the frequency response of female *Eristalinus*, shown in Figure 3.7. The frequency response to the chirp stimulus is shown in Figure 3.8. For the intact fly between 2–12 Hz, the head-roll gain dropped from around 0.6 to 0.4 as the stimulus frequency was increased. This matches the behaviour observed for the horsefly in Section 3.1.3. Above 12 Hz, the gain increased linearly, with a peak gain at the highest frequency analysed in the chirp stimulus—just below 20 Hz. The phase angle of the head rotations decreased linearly between 2–20 Hz, dropping from approximately $-20^\circ$ to $-90^\circ$. Tested in the dark, almost the same frequency response was found: only a small reduction in gain (<0.1) be-
tween 2–6 Hz was observed. At frequencies above 12 Hz the gain increased, unaffected by the lack of visual input. After removing the halteres, a further small decrease in gain was observed in the low, 2–6 Hz range, and a greater decrease, to almost no response in the intermediate range, 6–11 Hz. At higher frequencies, the gain increased, with an almost identical response to the intact condition.

Within the frequency range tested with the chirp, it is apparent that the compound eyes and halteres only contributed to head-roll responses up to 12 Hz. Above this, the head appears to have been stabilised by its inertia. However, the absolute values obtained by the chirp response in Section 2.3.1 were questioned when presented. To validate these findings, and to sample the lower frequencies which were not captured in the chirp response, a second frequency response was obtained using constant frequency sinusoidal stimuli over a logarithmically-spaced range from 0.01–25 Hz.

3.2.6 CFS frequency response, *Eristalinus aeneus*

The frequency response to the CFS stimuli, shown in Figure 3.9, covers a greater range at both low and high frequencies than that obtained with the chirp stimulus. Encouragingly, the two plots show very similar gain and phase of responses for the range in which they overlap, between 2–19 Hz. The gain at 20 Hz was found to be slightly lower than estimated from the chirp response, although still almost identical across each of the conditions tested. Furthermore, a plateau in the gain is revealed, with a similar response even at 25 Hz.

A clearer separation between the response gain in the dark condition and the illuminated setup was found for the CFS frequency response. The compound eyes, and possibly also the ocelli, account for approximately a quarter of the response between 1–12 Hz. At lower frequencies, they seem to contribute less than the halteres, although between 0.01–0.3 Hz the response was low in all conditions. Here, the head generally followed the thorax, as shown in the first 1.5 seconds of Figure 3.5.
3. Head-roll stabilisation in three Dipteran families

**Figure 3.8:** Frequency response of *Eristalinus aeneus* head-roll response to chirp stimulus. The plot shows the average of the response to an increasing and a decreasing chirp rate (see Section 2.3.1 for details). Mean response gain (top) and phase (bottom) of $N = 5$ flies, error bars show ± standard error.

**Figure 3.9:** Frequency response of *Eristalinus aeneus* head-roll response to constant frequency sinusoidal thorax rotations. Mean response gain (top) and phase angle (bottom) of $N = 5$ flies, error bars show ± standard error.
The decrease in gain resulting from the loss of haltere input was found to be greater in the CFS frequency response. The head response also appeared to depend on the halteres up to higher frequencies: the mean gain was approximately halved in their absence at 15 Hz, although a large error bar at that frequency indicates a substantial variability in the responses across individual flies. At 20 and 25 Hz, the loss of halteres had almost no effect on the gain, and across all frequencies no change in the phase of the response occurred.

### 3.2.7 Retinal slip speeds

To assess the effectiveness of the roll stabilisation employed by *Eristalinus*, the retinal slip speeds were calculated, as in Section 3.1.4, and plot in Figure 3.10 (top).

![Retinal slip speed](figure310.png)

**Figure 3.10:** Retinal slip speed in the hoverfly *Eristalinus aeneus*. Top: Mean probability density function (PDF) of the slip speeds experienced by the hoverfly during CFS thorax-roll at four frequencies, which show the contribution of the halteres. Bottom: The median slip speed across the entire frequency range tested. N = 4 flies, error bars show ± standard error.
For both the intact condition and the condition with the halteres removed, the distribution peaks were shifted to lower slip speeds than the peak thorax-roll velocity (grey vertical line), and the increased effectiveness at higher frequencies is clearly visible. The bottom plot in Figure 3.10 presents the median of these distributions and further clarifies the performance at different frequencies, and the contrast with similar plots for the blowfly and horsefly, shown in Figure 3.3. At frequencies up to 10 Hz, the stabilisation is similar to that of the blowfly and robberfly; slip speed increased linearly with stimulus frequency, and was further increased when tested without the halteres.

Free-flight video showed high roll angles in flight, with a steady gaze, as in Figure 3.6; as a strategy for allowing thorax-roll disturbances during hovering, while keeping the head level, the passive inertial stability proposed here would appear to cope well with very high angular velocities.

3.2.8 Discussion

The head movements of three species of hoverfly in response to thorax-roll rotations were quite different to the typical stabilisation behaviour of blowflies. From the movement of the head, the neck motor system appeared to be in one of two states: locked, so that the head and thorax rotated together, or loose, so that the head was largely uninfluenced by the motion of the thorax. In the loose state, the head could be either stabilised by its inertia, and show little rotation, or follow the thorax with a long delay, as if the head and thorax were connected by a spring with low torsional stiffness. This was apparent in each of the hoverfly species tested, with different sizes and body shapes. The same effect was not observed in any of the other fly families examined in this chapter, nor in preliminary experiments on beeflies (*Bombylius major*, results not shown), which are often seen hovering and making chasing flights in a similar manner to hoverflies.
A form of inertial gaze stabilisation has been shown in dragonflies (Mittelstaedt, 1950). The ‘head-arrester’ system of the dragonfly enables mechanical locking of the head to the thorax, via an arrangement of sclerites at the neck-joint which protrude into the head capsule. Upon releasing the head from the arrested state, the neck is free to rotate, aided by folds in the structure of the surrounding membrane which impart a high degree of flexibility (Gorb, 2000). From observations of free-flight, the head appears to be arrested during behaviours such as feeding or tandem flights which are performed at low velocities (Gorb, 1999); agile flight is associated with the head in the un-arrested state, in which it can be stabilised.

The movements observed here in hoverflies bear a resemblance to the action of the dragonfly head-arrester system and inertial stabilisation. At low velocities (0.01–0.3 Hz) the head could be seen to move with the thorax (illustrated in Figure 3.5), corresponding to the arrested state. At intermediate velocities (1–10 Hz), the stabilisation appeared to be under sensory feedback control, as the different experimental conditions were shown to impair performance. In this regime, gaze stabilisation resembled that of other Dipteran flies.

At high velocities (15–25 Hz), the head appeared to be stabilised by its inertia—increasing frequency and excluding sensory input had little observable effect on the gain or phase of the head response. A high degree of flexibility in the hoverfly neck was observed, resembling that of the dragonfly. The head in the loose state also often accumulated errors. The head of Episyrphus was observed numerous times at an offset of around 90° for a few seconds, particularly after rapid steps in rotation of the thorax. Step results were not presented here, however in similar experiments, Goulard et al. (2015) applied a step rotation to the thorax and observed an overshoot and sustained uncorrected error which confirms this finding.

The neck motor system of the blowfly exhibits elasticity which provides a passive restoring force to correct for such static offsets of the head (Gilbert and Bauer,
In *Episyrphus*, at least, this elasticity appears to be absent. Goulard et al. observed compensatory head movements which were in line with the stabilisation observed here in the intermediate frequency range, and therefore similar to the behaviour in blowflies. In order to account for stabilisation without visual input, the authors hypothesised proprioception in the neck which could detect and correct for deviations in thorax-head angle, but could not explain the static error they also occasionally observed. It seems plausible that in a loose-head state the suggested proprioceptors would cease to detect head-roll.

Another intriguing result found by Goulard et al. was the higher response gain of male hoverflies and the higher variability in their response, compared to females. Different behavioural states have been described in female blowflies, which were shown to exhibit a change in head response gain depending on internal states (Rosner et al., 2009), and although Goulard et al. do not rule this out, flight and gaze behaviour of hoverflies is known to differ between the sexes (Collett and Land, 1975). Inertial stabilisation of the head could be activated during particular bouts of flight, for example during saccades, when optomotor reflexes are overruled by voluntary turns. Hovering and forward flight may induce a shift in the method of gaze stabilisation. With the equipment used here, flight could be classified by analysing wing stroke amplitude and kinematics, or the position of the alula, an external membrane close to the wing base which is associated with distinct flight modes (Nalbach, 1989; Walker et al., 2012).

The existence of the head-arrester system in dragonflies and the evidence that it allows a passive stabilisation of the head in flight lends weight to the theory that hoverflies could also stabilise their gaze in this way. In dragonflies, a passive system may compensate for their lack of halteres, but it also appears to be functionally relevant during other behaviour, such as eating and mating (Gorb, 1999).
To further demonstrate that the inertia of the head alone can lead to the behaviour observed in hoverflies, the response of a previously developed mathematical model of the gaze stabilisation system was simulated without sensory input or motor output (Sabatier et al., 2014). The head and thorax were modelled as two rigid bodies, connected by a spring and damper, with the spring constant decreased by a factor of 10 from the value used to model the blowfly neck. The response to the chirp stimulus, shown in Figure 5.2, qualitatively confirmed that the passive, mechanical properties of the head could, with a flexible neck, reduce the amplitude of head rotation relative to the thorax.

**Figure 3.11:** Model response to chirp stimulus. The response of the modelled gaze stabilisation system to a simulated chirp stimulus applied to the thorax (grey trace).
3.3 Robberfly

3.3.1 Introduction

Robberflies (Asilidae) are predatory flies that hunt from the ground or from perches. Once a target is spotted, they launch interception flights towards their airborne prey, which can include the hoverflies and blowflies studied here, regardless of their relative size difference. They catch their prey in the air, inject them with neurotoxin and digestive enzymes, then carry them to a safe location to feed on the internal fluids (Stubbs and Drake, 2001).

Very little work has been carried out on the gaze stabilisation in this family of flies. The neural apparatus of robberflies seems to be quite different to other Dipteran flies in that no typical VS-like cells have been identified, which detect pitch and roll rotations in the blowfly (Buschbeck and Strausfeld, 1997). HS-like cells have instead been found at levels in the lobula plate where VS cells should be expected, suggesting a particular importance placed on detecting translation and yaw in flight. These differences may be related to body type and stability in the air; asilids are long-bodied and as well as performing interception flights, some species are also known to hover for periods.

3.3.2 Roll behaviour experiments

*Philonicus albiceps*, commonly known as the dune robberfly, was chosen for this study for its comparable size to *Calliphora*, and because it could be reliably found in dunes where it hunts. Robberflies were caught in Camber Sands, East Sussex, and returned to the laboratory on ice. Flies were kept with sugar and water and a supply of live blowflies. When kept near a window for light, flying prey could be caught within a net cage (approximately 50 cm × 20 cm × 20 cm). Flies did not survive for more than a week in captivity.
3. Head-roll stabilisation in three Dipteran families

For experiments, flies were tethered following the method described in Section 2.2.1 and mounted on the step motor. In the version of the setup used for robberflies, the camera was angled downwards to view the fly from above, which be seen in Figure 3.12. The estimation of head angle from the video analysis (see Section 2.2.4) incurred an error from this perspective. This was calibrated using a head-fixed fly and a correction was applied to all head-roll traces. All other components of the setup, including lighting conditions, were the same as previously described.

Figure 3.12: Photograph of a female robberfly, *Philonicus albiceps* (Courtesy of Steven Falk) Right: Video frame of roll behaviour experiment.

3.3.3 Frequency response

Constant frequency sinusoidal stimuli with an amplitude of 30° were applied to the thorax at 1 Hz for 20 seconds, and at 3, 6 and 10 Hz for 10 seconds. Three trials were run for nine robberflies and then repeated after occluding the ocelli. For a different set of eight robberflies, and on different days, the same trials were run without halteres. This allowed the contribution of the ocelli to be examined with and without the haltere input.

Robberflies did not fly readily while tethered. Flight was often interrupted mid-trial—it was necessary to segment videos by flight activity, and only those sequences with flight were analysed. For more than half of the flies, airflow alone was not
sufficient to encourage flight. An artificial perch was made which could be removed shortly before trials began and this proved to be reliable for initiating flight.

![Frequency response: robberfly](image)

**Figure 3.13:** Frequency response of the robberfly head to thorax-roll rotation. Mean head-roll gain (top) and phase (bottom), for \( N = 9 \) robberflies with halteres (green) and \( N = 8 \) robberflies without halteres (purple), \( n = 2 \) trials, error bars show ± standard error.

On inspection of the video footage, head responses appeared to show little stabilisation of the head. However, averaging over trials and flies revealed a peak head response which compensated for thorax-roll with an average gain of 0.6 at 1 Hz, for the intact robberfly. As frequency was increased, the gain was reduced—to 0.4 at 10 Hz. The phase delay was also extended, but remained within \(-20^\circ\) of the phase of the stimulus. At 1 Hz, the gain of the response was unaffected by the occlusion of the ocelli and the halteres. A slight increase in mean gain can be seen in the response at 1 Hz, however this is within the standard error of the estimated mean intact response, and does not appear to be significant. The phase of the response was shifted by approximately \(-15^\circ\) without ocelli and without halteres, to give a slower response relative to the thorax. At higher frequencies, this shift increased for both conditions without halteres, reaching \(-90^\circ\) at 10 Hz. The gain at 10 Hz for
these conditions was between 0.2–0.3, while the gain with the halteres was only around 0.05 higher.

### 3.3.4 Ocelli effect gain and phase

At frequencies higher than 1 Hz—the peak response—the robberfly gaze stabilisation system output head movements with lower amplitude, indicating decreased performance. At these challenging frequencies, the contribution of the individual sensory modalities could be seen. Interestingly, the contribution of the ocelli to the gain of the system appeared equal to the contribution from the halteres, suggesting a more prominent role for the ocelli than the results in Section 2.3.2 imply for the blowfly. Furthermore, occluding the ocelli only had a negative impact on phase when the halteres were removed. This could imply a separate gating of the ocellar pathway by the haltere signal, similar to the haltere-dependent gating of visual signals in blowflies (Huston and Krapp, 2009).

### 3.3.5 Discussion

Unlike the horseflies studied, robberflies were not cooperative in these experiments. Whether due to low temperature or lighting conditions compared to their habitat—Philonicus albiceps were not seen flying in the wild on overcast days—or restriction of their movement by the tether and fixation used, the robberflies did not fly willingly. Their low response amplitudes could have been due to this; flies trying to escape their situation would not be expected to respond to an externally forced body rotation in tethered flight the same way they would under natural conditions. Different gain states could also have been responsible, as discussed for hoverflies in Section 3.2.8, although no obvious clustering of responses was found. Despite this, by averaging over a greater number of animals compared to other species, robberflies
did make compensatory movements of the head in response to thorax-roll perturbations, and the contribution of their halteres and ocelli could be discerned from their head responses.

With no known VS-like tangential cells in robberfly species studied, the neurons involved in processing rotational optic flow from compound eye input remain to be found (Buschbeck and Strausfeld, 1997). The robberfly appears to be capable of stabilising roll rotations with compound eye input alone, although without the corresponding experiments in the dark this has not been proven explicitly here.

In Section 2.3.2, the occlusion of the ocelli was shown to have almost no impact on the blowfly head response, whereas clipping the halteres had a much greater effect on both the gain and phase. For the robberfly, the loss of ocellar input caused a decrease in gain comparable with the loss of the halteres. This suggests a more important role for the ocelli in the robberfly gaze stabilisation system than the blowfly. The role of the ocelli in both of these insects is investigated further in Chapter 4.
4. Integration of visual pathways in the blowfly

This chapter focuses on the interaction between the two visual systems of the blowfly, *Calliphora vicina*, and their contribution to gaze stabilisation reflexes. In Section 4.3, behavioural experiments in the blowfly reveal a subtle effect of the ocelli in reducing the response delay of head movements which compensate for high frequency rotations of a visual horizon. Control of these head movements is shown to employ a non-linear combination of ocellar and compound eye information. This finding motivated electrophysiological recordings of VS and descending neurons, presented in Section 4.4, in order to understand the integration of the two visual pathways within the nervous system.

4.1 Introduction

The role of the ocelli in the gaze stabilisation reflex of blowflies remains somewhat unclear. For other insects in which ocelli have been studied, such as locusts and dragonflies, they contribute directly to head movements (Wilson, 1978). The fast transduction of their signals results in the neck motor system receiving ocellar input before the arrival of the compound eye signal and, when combined, the response delay of the head is reduced (Taylor, 1981). Flies possess ocelli positioned dorsally, rather than frontally, and they appear to be structurally and functionally different. Amongst the insect ocelli studied thus far, they require comparatively high light levels to operate, but are highly sensitive to fast luminance changes and give a rapid response (Mizunami, 1995). Schuppe & Hengstenberg (1993) found minimal contribution of the ocelli to head movements in blowflies when using a stationary or 1 Hz sinusoidal motion stimulus, whereas with a 10 Hz oscillation, Parsons (2008) observed an effect on the latency and amplitude of head responses. However, neither study tested responses over a range of frequencies, which could confirm that the
4. Integration of visual pathways in the blowfly

behavioural effect of the ocelli is dependent on the stimulus. Furthermore, the independent ocellar contributions were found by subtracting response traces, disregarding the dynamic interplay between the sensors as part of a feedback system.

Here, the gaze stabilisation behaviour is examined at a range of frequencies, and an automatic control theory approach is used to probe the contribution of the ocellar pathway. Electrophysiological recordings show the response of two neurons along the gaze stabilisation pathway, at the site of integration of the ocellar and compound eye signals.

4.2 Materials and methods

4.2.1 Behavioural experiments

To examine the contribution of the visual systems alone required the elimination of haltere input, and other thorax-based mechanosensors that could possibly detect roll rotations. The experimental setup followed the description in Section 2.2, with a modification to confine the fly to a fixed position and instead rotate the artificial horizon around it. Thus, the stimulus was purely visual. As before, the stimulus time-series was a sinusoidal roll rotation with an amplitude of $30^\circ$, and frontal airflow was applied continuously to encourage flight. After testing the response to rotations at 1, 3, 6, and 10 Hz, the ocelli were occluded with black paint and the same experiments were repeated, providing visual input only to the compound eyes.

4.2.2 Linear feedback model

A linear model of the gaze control system was proposed, incorporating only visual sensors. Figure 4.1 shows a block diagram of the model. The visual error that resulted from movement of the horizon is termed HA. This is mathematically equivalent to the absolute angle of the head, HA, measured in experiments in Chapters 2 and 3. Here, the head angle measured from video footage also corresponds to the
4. Integration of visual pathways in the blowfly

relative angle of the head to the thorax, since the thorax remains stationary, and the convention previously established for this term, HR, is kept.

![Figure 4.1: Linear feedback model. Left: block diagram of control system. Outputs from the transfer functions of the compound eyes, $G_{CE}$, and the ocelli, $G_O$, are combined linearly. Right: conventions used for behavioural experiments.](image)

Testing of the intact fly provided data for the output of the system, HR, while the transfer functions of the compound eyes, $G_{CE}$, and the ocelli, $G_O$, were treated as a ‘black-box’ to be investigated. Informed by the anatomy of the visual systems, their convergence at descending neuron terminals (Strausfeld and Bassemir, 1985), and evidence from previous electrophysiological studies (Wertz et al., 2008), the two sensors were assumed to operate in parallel with their outputs linearly combined:

$$G_{CE}(s) + G_O(s) = \frac{C_1(s)}{1-C_1(s)}$$

where $C_1$ represents the sampled behavioural response for the intact fly, and $s$ is the Laplace variable. Under condition $C_2$, the ocelli were occluded. This eliminated input to $G_O$, allowing the compound eye pathway to be studied in isolation:

$$G_{CE}(s) = \frac{C_2(s)}{1-C_2(s)}$$

Blowflies did not tend to fly with the compound eyes occluded, so $G_O$ could not be examined in the same manner. However, once the open-loop transfer function of the compound eyes had been found, $G_O$ could be examined:

$$G_O(s) = \frac{C_1(s)}{1-C_1(s)} - \frac{C_2(s)}{1-C_2(s)}$$
4. Integration of visual pathways in the blowfly

4.2.3 Electrophysiology

The experimental setup used to test the head-roll behaviour was replicated as closely as possible, with modifications which allowed for intracellular electrophysiological recordings to be performed (Figure 4.2). The extent of the artificial horizon was reduced by 60° in the posterior visual field in order to accommodate a platform on which the fly was held. The other major differences with the behavioural experiments are that the fly was not in tethered flight, and that the head was not free to move. This resulted in an open-loop condition of the gaze stabilisation system, whereas the behavioural experiment was in closed-loop.

Experiments were conducted on different animals to the behavioural experiments, from the same colony. 1–3 day old female *Calliphora vicina* were cooled with ice for 5–10 minutes to reduce movement. The legs, wings and proboscis were removed, and the wounds sealed with beeswax. Flies were then fixed to a holder and their heads positioned using the deep pseudopupil technique (Franceschini, 1975) to align the equator of the compound eye with the horizontal of the laboratory reference frame. An incision was made in the neck membrane and the gut was removed to reduce movement of the brain induced by its contractions, and to allow access to the neck-connective neural tissue. For recordings made in the lobula plate, openings were made in the cuticle at the rear of the head capsule on both sides. Air sacs and major trachea covering the lobula plate were removed on the fly’s left side.

Electrodes were fabricated on a Flaming-Brown filament puller (P1000, Sutter Instruments, USA), using thick-walled, borosilicate glass capillaries (GC100F-7.5, Clark Electromedical Instruments, UK). 2 M potassium chloride was used as an internal conducting solution, giving electrodes a resistance of 20–40 MΩ. For staining neurons, the tip of the electrode was filled with 5 mM Alexa 488 fluorescent tracer (Invitrogen, USA) in 2 M potassium chloride. A blunt tungsten electrode was inserted into the right side of the head capsule to act as a reference. Physiological
signals were measured using an SEC-10LX (NPI Electronic, GmbH) amplifier operating in bridge mode and sampled using a USB-6211 (National Instruments) analog-digital converter at 20,000 samples/s. The stimulus frequencies used for behavioural experiments were presented in a pseudorandom order for 2 seconds each, with an interval of 0.5 seconds during which the horizon remained level.

![Figure 4.2: Setup of electrophysiology experiment. The motor drives the horizon here while the fly is stationary. An electrode could be inserted into the lobula plate or neck-connective to record activity in response to a visual stimulus equivalent to the one used in the behavioural experiments.](image)

**Figure 4.2:** Setup of electrophysiology experiment. The motor drives the horizon here while the fly is stationary. An electrode could be inserted into the lobula plate or neck-connective to record activity in response to a visual stimulus equivalent to the one used in the behavioural experiments.

### 4.3 Behavioural results

#### 4.3.1 Blowfly head-roll response

Experiments were conducted on eight blowflies. On average, the head was observed to make rotations in the same direction as the stimulus, with a phase delay of at least one eighth of a cycle, and an amplitude of less than one half of the stimulus amplitude. The results are shown in Figure 4.3. The movements minimised the angle between the head and the horizon. At higher frequencies, the amplitude of the response decreased and the effectiveness of the compensatory head movements was thereby reduced. After occluding the ocelli, the individual cycle responses showed little difference in terms of amplitude or delay.
Figure 4.3: Blowfly head-roll response traces. A: Example trace obtained from one fly, extracted from video data. Roll angle of head (bottom) follows the visual stimulus angle (top). B: Mean and SEM of behavioural responses to single cycles of the stimulus for the intact condition, blue (top), and with the ocelli occluded, orange (bottom). Obtained from 136 cycles at 1 Hz and 752 cycles at 10 Hz, N = 8 flies. The amplitude of the head movements was smaller at 10 Hz than at 1 Hz, whereas little difference was apparent between the two conditions.

4.3.2 Blowfly frequency response

The gain of the frequency response was taken to be the ratio of the head response, HR, to the horizon roll angle, HA. When plot against frequency, shown in Figure 4.4, the mean gain across animals dropped from around 0.35 at 1 Hz, to below 0.1 at 10 Hz. The gain was found to be almost identical after occluding the ocelli. At each frequency, the gain was a least 0.3 units lower than the response obtained using thorax-roll rotations, reproduced in Section 2.3.5, either with or without intact halteres. Some of the potential causes for the gain remaining the same under both conditions will be discussed in Section 4.5. Overall, the trend with increasing frequency was found to be qualitatively similar to the results described in previous chapters.
The phase of the response showed a very slight difference between the two conditions, with a general shift towards longer delays when the input to the ocelli was excluded. Interestingly, this did not hold to be true at 1 Hz, where the phase advanced slightly. A two-tailed Wilcoxon rank sum test showed the phase difference resulting from occlusion of the ocelli to be significant at 1, 6 and 10 Hz. These results are summarised in Table 4.1, using median estimates where the data were not normally distributed.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Median C1 delay [ms]</th>
<th>Delay shift without ocelli [ms]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Hz</td>
<td>64</td>
<td>−12</td>
<td>0.017</td>
</tr>
<tr>
<td>3 Hz</td>
<td>34</td>
<td>0</td>
<td>0.098</td>
</tr>
<tr>
<td>6 Hz</td>
<td>30</td>
<td>+8</td>
<td>&lt;10^{−13}</td>
</tr>
<tr>
<td>10 Hz</td>
<td>24</td>
<td>+4</td>
<td>&lt;10^{−10}</td>
</tr>
</tbody>
</table>

Table 4.1: Head-roll response delay attributed to the ocelli.

Figure 4.4: Frequency response of the blowfly head to a roll rotation of the visual horizon. Body-fixed, visual input only. Mean head-roll gain (top) and phase (bottom), for N = 8 flies, error bars show ± standard error. With ocelli occluded, the phase delay shows significant difference (p < 0.05) at 1, 6, and 10 Hz.
It is clear that occluding the ocelli reduced the response delay at the two highest frequencies, and these shifts were more statistically significant than at the two lower frequencies. 3 Hz appears to be the crossover, at which point the ocelli had no effect on phase. At 1 Hz, the phase underwent the largest shift after occluding the ocelli, but towards a shorter response delay. Previous studies in which similar experiments were conducted at 1 Hz found no effect of the ocelli on head-roll, so a difference was not expected here. It is worth noting that the median delay at 1 Hz for the intact fly was nearly twice as long as the delay at higher frequencies. It was also longer than the delay after occluding the ocelli at those frequencies, so the temporal response at 1 Hz with both visual systems was slower than at higher frequencies with only one.

4.3.3 Individual pathway responses

The open-loop transfer function for the compound eyes was found as described in Section 4.2.2. The gain and phase for the compound eye pathway were largely similar to the behavioural response C2, with compound eye input alone. The estimates of these values are shown for each individual fly in Figure 4.5. For the ocellar pathway, the gain plot follows a similar trend to the closed-loop behavioural output, with a lower magnitude which suggests that, as expected, the compound eyes made a greater contribution to the overall response amplitude. However, the phase data from individual flies were distributed fairly evenly over the range of 0–360°. That is to say, the phase of the ocellar pathway showed little correlation with the behavioural output.
These results are problematic for two reasons. Firstly, the open-loop gain of the ocelli was found to be non-zero, so their removing their input should have had some impact if the two pathways were indeed combined linearly. However, no difference in gain was observed in the behavioural experiments upon ocellar occlusion. Secondly, the open-loop phase of the ocelli implies that the behavioural output, while dependent on the ocellar response in terms of gain, is completely insensitive to its phase. This seems unlikely—such a system would essentially respond randomly to visual input and would degrade the accuracy of the output from the compound eye pathway. It is intriguing that this would explain the findings at 1 Hz, whereby occlusion of the ocelli caused a faster response, however the same was not found to be true at other frequencies. Rather, the assumption of a linear combination of the two pathways is more likely to be incorrect, and there appears to be a context-dependency of the behavioural response, which is discussed further in Section 4.5.
4. Integration of visual pathways in the blowfly

4.3.4 Robberfly head-roll behaviour

The same experiments were also carried out with the robberfly, *Philonicus albiceps*, to investigate whether a linear summation of the two visual systems would predict the behavioural output in other species. It was quickly seen that the robberflies did not respond well to the stimulus, and appeared to make little attempt to stabilise against the rotating horizon. The results for three flies are shown in Figure 4.6. During the experiments, the correlation of head-roll behaviour with the stimulus was not apparent and tests were not taken further. However, from the mean cycle responses, occasional very small deflections in the direction of each stimulus rotation were seen for the experiments at 10 Hz.

The gain of the robberfly frequency response, shown in Figure 4.7, was shown to be low compared to that of the blowfly, showing little change across frequencies. The phase of the response at 1 Hz for the condition with both compound eye and ocellar input showed wide variability, indicating little correlation with the phase of the stimulus. At 3 Hz and above, the phase showed a linear decrease with increasing frequency, and a subsequent phase shift downwards was seen for the condition with compound eye input only. This indicates that occlusion of the ocelli in the robberflies caused a similar increase in delay at high frequencies as observed in the blowflies.

The mean gain of around 0.1 at all frequencies tested corresponded to a response of approximately 3° to a 30° rotation of the stimulus. This was not considered to be a stabilisation behaviour of the type seen for the blowfly and other flies studied in Chapter 3. Given this, and the advantages of studying blowflies from a colony in terms of ease and abundance of animals, further investigations into the effect of the ocelli via electrophysiology were not carried out on robberflies.
Figure 4.6: Robberfly head-roll response traces. A: Example obtained from one fly, extracted from video data. Roll angle of head (bottom) shows little correlation with visual stimulus angle (top). B: Section of a trial at a higher frequency, showing low amplitude head movements. C: Mean and standard error of behavioural responses to single cycles of the stimulus for the intact condition, green (top), and with the ocelli occluded, purple (bottom). Obtained from 55 cycles at 1 Hz and 297 cycles at 10 Hz, N = 3 flies.

Figure 4.7: Frequency response of the robberfly head to a roll rotation of the visual horizon. Body-fixed, visual input only. Mean head-roll gain (top) and phase (bottom), N = 3 flies, error bars show ± standard error.
4. Integration of visual pathways in the blowfly

4.4 Interneuron electrophysiology

The findings that the combination of visual pathways does not appear to be linear, and that the response delay seems to depend on the frequency of stimulation, prompted a search for similar evidence within specific interneurons known to constitute these pathways. The activity of VS cells in the lobula plate and descending neurons were recorded in the blowfly in response to the same rotating horizon stimulus used to examine gaze stabilisation behaviour. The connectivity of these neurons is shown in Figure 4.8. The VS cells process retinotopic input from the compound eyes and have been shown to respond in a direction-selective manner, with receptive field tunings which resemble matched-filters for rotational optic flow (Krapp and Hengstenberg, 1996). They are hypothesised to contribute to roll gaze stabilisation reflexes, with VS4–7 most sensitive in the lateral visual field, which corresponds to the motion encountered during clockwise roll rotations of the fly. These were therefore targeted to investigate the output of the compound eye pathway, represented by $G_{CE}$ in the analysis of the behavioural experiments.

Downstream of the lobula plate, three identified descending neurons receive input from a subset of the VS cells and the L-neurons, which convey ocellar output. These cells, referred to as DNOVS1–3, for ‘descending neuron of the ocellar and vertical system’, are candidate sites for the integration of the two visual systems. Although their response properties have previously been investigated in the blowfly, they have not been characterised at frequencies as high as those used in the behavioural experiments here, nor with a stimulus which covers the receptive fields of both the compound eyes and ocelli. The descending neurons were therefore targeted to study the way in which the two visual pathways are combined.
4.4.1 VS and DNOVS responses

Recordings were made from a VS cell, identified from its anatomy after dye injection to be VS5 (Hengstenberg et al., 1982), and a descending neuron, suspected to be DNOVS1 from its non-spiking response and sensitivity in the lateral visual field (Haag et al., 2007). Both cells were predominantly sensitive to vertical motion over
4. Integration of visual pathways in the blowfly

the ipsilateral compound eye. The average response of each cell to a single cycle of
the stimulus is shown in Figure 4.9. The responses were normalised to the cycle
period at 1 Hz to facilitate comparison between frequencies, shown in Figure 4.10.
Both cells were depolarised during the downwards motion of the horizon across the
ipsilateral compound eye, followed by a hyperpolarisation during the upwards move-
ment in the second half of the cycle. The phase delay appeared to be on the order
of 20 ms in both neurons, and response amplitudes were approximately ±5 mV.

![Figure 4.9: VS and DNOVS responses. Mean change in membrane potential (base-
line subtracted) for a single cycle of the horizon roll stimulus, starting at +30° at
time = 0 and initially moving downwards on the ipsilateral side. Left: recording of
VS cell, visually identified as VS5, N = 1 blowfly, n = 7 trials, showing mean of
between 14 cycles (1 Hz) and 140 cycles (10 Hz), error bars show ± standard devi-
ation. Right: mean response of a descending neuron, suspected to be DNOVS1, for
N = 1 blowfly, n = 16 trials, error bars show ± standard deviation.]

Recording from the descending neurons proved to be challenging; from approxi-
mately 50 attempts, 3 were recorded successfully. A single stable recording was
achieved for a sufficiently long duration to record the response to the stimulus mul-
tiple times, and this was repeated for around 15 minutes. VS cell recordings were
successful for the majority of attempts, however the responses from a single animal
were presented here to provide a comparable estimate of the variability of a single
cell’s response.
The results from one animal cannot be interpreted with any certainty, and it would be premature to draw conclusions about function from this single sample. Nevertheless, both neurons responded consistently to the stimulus and showed little signal variation. The main difference in the normalised responses of the VS cell and descending neuron is illustrated in Figure 4.10: when stimulated at 6 and 10 Hz, a rapid rise in membrane potential occurred during the last quarter of the mean VS

**Figure 4.10:** Normalised VS and DNOVS responses. Mean responses to a single cycle of the sinusoidal stimulus, with each cycle normalised to the scale of the 1 Hz cycle period. The stimulus reversed direction at phase = $\pi$ radians.
response cycle, after the maximum hyperpolarisation of the cell. This response resembles what would be expected if the stimulus were moving in the opposite direction, as at the start of the cycle. The VS cell appeared to become more rapidly hyperpolarised than the descending neuron, but at 6 and 10 Hz this hyperpolarisation did not persist, and rapidly returned to baseline before the start of the next cycle. At both frequencies this feature was absent in the comparable response of the descending neuron, which resembled a low-pass filtered version of the VS cell response.

4.5 Discussion

4.5.1 Ocellar effect on head-roll delay and amplitude

In this chapter, behavioural experiments where performed on blowflies using a visual stimulus which mimicked the relative rotation of the ground and horizon during a roll rotation. Removing input to the ocelli increased the phase delay of compensatory head movements by an average of 6 ms at the two highest frequencies tested—approximately 20% of the overall delay. Although a seemingly small change, the response delay of the haltere mediated response is approximately 5 ms, so relative to the time-scales within the fly gaze stabilisation reflex, such an increase in speed does seem relevant (Taylor and Krapp, 2007). At an intermediate frequency of 3 Hz, the occlusion of the ocelli had no effect, and at the lowest frequency tested, 1 Hz, the response delay was decreased, by approximately 20% of the overall delay.

Removing the input to the ocelli had an effect on the phase of head movements, but no noticeable effect on their gain. This finding is in disagreement with the results obtained by Parsons (2008) with similar experiments at 10 Hz, which showed a decrease in gain and phase after occluding the ocelli. Why was the gain of head movements not affected by occluding the ocelli here? One possible answer could be the difference in experimental lighting conditions; Taylor (1981) found that both
the gain and phase of locust head movements was affected by cauterisation of the ocelli when tested in dim light, whereas at higher light intensities only the phase was affected. The luminance in Parsons’s experiments was significantly higher than in the experiments described here—30,000 versus 500 Cd/m²—and could explain the difference in behaviour. This would suggest that the blowfly’s ocelli contribute to the gain of head movements of blowflies in bright light, but not dim light—the opposite to the observation Taylor made for locust ocelli.

Schuppe and Hengstenberg (1993) found that the ocelli had little effect on stabilising head movements. In their experiments, like Taylor’s, the compound eyes were covered in order to evaluate the contribution of the ocelli alone and, for the blowfly, no response was observed. In the experiments presented here, and in Parsons’s study, the contribution of the ocelli was deduced from a comparison of responses which included compound eye input. Input from the ocelli alone is clearly not sufficient to drive compensatory head movements in the blowfly, as it is in locusts and dragonflies, but in combination with the compound eyes the ocelli do make a subtle contribution. This highlights the need to consider the impact that sensory modalities have on each other, as well as their individual contribution, when studying multisensory integration.

The amplitude of head movements in this horizon-roll setup was lower than observed for comparable experiments in which the thorax of the fly was rotated, which were partly reproduced in Section 2.3.5. This may indicate input from additional thorax-based mechanosensors, which were not stimulated in the experiments presented here. For instance, differential wing loading would have been substantially different between the two setups. Also, some neck-motor neurons have been shown only to respond to visual input when it coincides with haltere input and this may have also diminished the response amplitude (Huston and Krapp, 2009). Another
possibility, which was not ruled out, is that areas of visual contrast across the overhead light diffuser provided an additional motion cue in the thorax-roll experiments.

4.5.2 Behavioural results indicate non-linearity

The findings presented here point to a non-linear combination of outputs from the ocelli and the compound eyes, depending on light conditions and the frequency of visual stimulus rotation. Further support for this was provided by modelling a linear combination of the two pathways in a feedback system and demonstrating that the ocellar pathway response did not match the observed data. This was explored further within a descending neuron, which integrates input from both visual systems.

Remarkably, the descending neuron displayed bipolar responses conveying information about every individual cycle of the stimulus, with little reduction in response amplitude for stimuli up to 10 Hz. This is in marked contrast to the behavioural output. Assuming that the particular descending neuron recorded was part of the gaze stabilisation pathway, an ideal motor system with no response delay could be driven equally well in the 1–10 Hz range by the visual inputs alone. The response of the neck-motor system obviously does incur some delay, and this may be the cause of the decrease in behavioural output observed. A further processing stage may also be involved. The response of the descending neuron here appeared to show a loss of high-frequency information present in the presynaptic VS response. A further processing step could sample the descending neuron response with a lower temporal resolution, potentially matched to the capabilities and response time of the motor system.

Although no evidence for a significant non-linearity was found in the descending neuron response, a number of interesting features could be discerned nonetheless. Remarkably, the membrane potential of the DNOVS displayed bipolar responses correlated with downward and upward motion sections of the stimulus at oscillation frequencies as high as 10 Hz. With an average velocity of 1200°/s at 10 Hz, blurring
of spatial information would be expected in the output of the compound eye at these speeds, so it is surprising that these features are still captured in the neuronal response. In addition, very little reduction in the amplitude of the membrane potential’s response was observed as stimulus frequency was increased. This is in marked contrast to the behavioural output of head-roll rotations. The reduction in head-roll amplitude at high frequencies would appear to be due to a limitation downstream of the descending neurons—most likely the response time of the neck-motor system itself—and not due to a limitation of compound eye signal processing.

It remains to be seen how the descending neuron response would be affected by occlusion of the ocelli, and whether stimulus frequency and light intensity modulate the effect. The expected response to the dynamic horizon stimulus within the descending neuron is somewhat difficult to predict based on existing knowledge; VS cell responses are well-studied in response to motion stimuli under various light conditions but the ocellar L-neuron responses, from which the descending neurons also receive input, are understood less well. The L-neurons are known to hyperpolarise in response to increases in light intensity (Simmons et al., 1994; Haag et al., 2007), and various descending neurons, including DNOVS1, employ both chemical and electrical in their synapses with L-neurons (Strausfeld and Bassemir, 1985). Single L-neurons in bees have been shown to exhibit a range of response patterns, with both spiking and graded membrane responses observed depending on the stimulus (Milde, 1981). A similar variety of L-neuron activity may be responsible for the non-linear contribution of the ocelli to the head-roll behaviour seen here in blowflies—spiking activity has certainly been observed in some identified descending neurons in flies (Gronenberg and Strausfeld, 1992; Wertz et al., 2008).

One recent study could provide a clue as to why the input from the L-neurons is of opposite polarity to the input from the VS cells. Kim et al. (2015) showed evidence of an efference copy signal in Drosophila melanogaster during voluntary
4. Integration of visual pathways in the blowfly

body-rotations, which could negate the VS cell response that would otherwise be induced by self-motion. The L-neurons of flies have their cell bodies outside of the central nervous system, which is exceptional for interneurons and unlike the L-neurons of other insects (Strausfeld, 1976). It is conceivable that, given their location, an efference signal would not suppress the ocellar output provided by the L-neurons, and during rotations their signals would reach the descending neurons unaffected. In such a case, the response from the ocelli alone might not have the same influence as it would when coincident with the VS input—as indicated in the results presented here. Such an arrangement could thus avoid unintended stabilising head movements caused by stimulation of the ocelli during voluntary turns.
5. Concluding remarks

5.1 Summary

In this thesis, comparative work was presented, aimed towards understanding general principles of sensorimotor control in the context of an animal’s stabilisation reflexes. In quantitative behavioural studies, several input functions were tested and an experimental method was implemented which could be applied to characterise the multisensory gaze stabilisation system of different fly species. Experiments were performed on flies from four Dipteran families, exploring the integration and contribution of several sensory modalities.

In the introduction, the question was raised as to whether the variation in neuronal morphology in the lobula plate of Dipteran flies reflects similar variation in gaze stabilisation behaviour. No directly comparable, controlled investigation had previously been carried out to answer this, and the results presented here make initial steps towards understanding the relationship between these neurons and behaviour across Diptera. The flies studied here represent but a small sample of their phylogenetic relatives, as the order of Diptera comprises over 100 families with diverse flight behaviours and morphologies, shown in Figure 5.1 (Buschbeck and Strausfeld, 1997). From the small sample of behaviour investigated here, it would appear that gaze stabilisation—in terms of performance and the contribution of different sensory modalities—varies considerably in its ability to compensate for externally forced thorax rotations.

Of the four families studied, the greatest body of knowledge on multisensory integration of the gaze stabilisation system had been accumulated in blowflies. These were considered the ‘gold standard’ in the work presented here, although they had not been previously shown to be any more effective at stabilising their gaze than other species of fly. Here, the results showed that up to a rotational frequency of
5. Concluding remarks

10 Hz, or angular velocities of around 2000°/s, the blowfly, *Caliphora*, is indeed the most effective species. It was shown that at higher frequencies, all three hoverfly species tested exhibited a response of the head not previously reported in Diptera. The ‘loose’ state of their heads reduced the influence of thorax rotation on the head through a significant decrease in the stiffness of the neck. At high velocities, the head became mechanistically stabilised independently of visual and mechanosensory stimulation which, at lower frequencies, did contribute to the gaze stabilisation reflex, as in blowflies. This suggests that sensory feedback may be partially disabled in the high dynamic input range.

Clearly, at extremely high rotational velocities, the neck may exceed its biological safety factor, and the head may move unhindered, which could result in similar kinematics, but in these flies no noticeable effect was observed on subsequent head movements, for instance during grooming. A rudimentary model of the mechanics of the head during these rotations supports the premise that the inertia of the head could accomplish passive stabilization in this way. A highly similar stabilization has also been described in dragonflies, along with the mechanism of establishing a ‘loose’ head. Analogous features in the neck motor system of hoverflies would lend further evidence to support this observation, and work to search for these via x-ray micro-CT anatomical studies is ongoing.
5. Concluding remarks

**Figure 5.1:** Phylogenetic relationships between Diptera. Arrows indicate the four families investigated in this work. Modified from (Buschbeck and Strausfeld, 1997)

**Figure 5.2:** Frequency response of all flies tested. This comparative plot shows the intact response of four of the flies investigated in this thesis, in the common frequency range 1–10 Hz. For details, see Chapter 3.
5. Concluding remarks

Experiments on the tabanid fly, *Tabanus bromius*, showed for the first time that flies from this family also stabilise gaze, much in the same way as blowflies. Its performance at high velocities was only marginally lower than that of the blowfly, and it compensated for low velocities with a higher gain. This may reflect the need for stabilising slower roll disturbances as might be expected during long periods of straight-ahead flight in relatively calm winds. The absence of the dorsal ocelli was proposed as a possible explanation for the reduced performance at high velocities.

The asilid, *Philonicus albiceps*, showed arguably the most contrasting response to the blowfly, with a lower response gain than all other flies tested. However, these experiments were conducted under a somewhat forced flight condition. As such, its behaviour is unlikely to represent its gaze stabilisation behaviour in free-flight, but it can be reported that this fly does stabilise its gaze, and the qualitative contributions of the modalities could be seen. The robberfly ocelli, in particular, showed a quite different contribution to those of the blowfly and appeared to decrease the phase delay.

The role of the ocelli in these two flies was investigated individually, through the use of a visual-only roll stimulation. Perhaps unsurprisingly, robberflies showed an even lower response in this setup, which was hardly discernible from the noise level. For the blowflies, a non-linear integration of the visual systems was identified, along with a weighting to their contribution that may be dependent on ambient light levels. Both of these findings suggest a more detailed examination of the integration within the descending neurons is required, particularly in their response to high temporal frequency stimuli. Initial intracellular recordings to this end were performed, and in time they could reveal the basis of the non-linear interaction described.
5.2 Future directions

Here, the applications of the work described in each chapter are described with the future directions for expanding aspects of it.

Chapter 2. The chirp stimulus proved to give comparable results to CFS experiments when applied to the hoverfly and horsefly, and qualitatively comparable results for the blowfly. Further confidence in the protocol would be gained by understanding the mechanisms underlying the observed difference, which may include sensory adaptation. The time-efficiency of this stimulus makes it preferable to CFS stimuli, and where further behavioural experiments are conducted a chirp will be used.

The white noise stimulus was greatly hindered by the limitations of the step motor used. Replacing the motor with a higher specification version would enable an expansion of the meaningful range of frequencies that could be applied during the experiments. It was possible to demonstrate the contribution of different sensory modalities in blowflies using Gaussian-distributed random noise, and the results obtained were supported by findings with other stimulation functions. A comprehensive analysis using a white-noise stimulus with a higher bandwidth remains a viable option for future research.

Chapter 3. The future line of investigation, now that differences in gaze stabilisation behaviour and the supporting sensors have been found, would be to establish more specific links between neuronal function and behaviour. To explore this, receptive field properties of the LPTCs will have to be studied to test whether their preferred self-motion axes reflect species-specific modes of natural motion that
the animals need to detect and control. This could be achieved by comparing the set of preferred self-motion axes of the LPTCs with the flight dynamics models of the different species, the latter of which could be based on the analysis of free-flight data or the measurements of forces generated by the flight motor under tethered flight conditions.

Anatomical investigations of the neck motor system will greatly aid the characterisation of muscle pulling planes and their effect on the head. These would be of fundamental importance in identifying the organization of the coordinate system used in the sensorimotor transformation of the gaze stabilisation system.

Chapter 4. The next steps in the investigation of visual signal integration in descending neurons is clear: further supporting electrophysiology should elucidate this integration. From there, a more informative comparison of the ocellar contribution could be carried out across species.


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Bibliography


