Chasing control in male blowflies: Behavioural performance and neuronal responses.

Dissertation

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Erklärung

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Contents

1 Summary

In the context of mating behaviour male flies pursue other flies in very fast visually guided chasing flights. During chasing they reach yaw velocities of up to 5000°/s. Chasing behaviour that can hardly be followed by the human eye belongs to the fastest visually controlled behaviours that can be found in nature. Therefore, the underlying physiological mechanisms are of great interest. Within the scope of my thesis I investigated the control of the chasing behaviour at two levels, the behavioural level and the neuronal level. On the basis of a quantitative analysis of the behaviour of freely flying blowflies (*Lucilia spec.*) I draw conclusions about interactions of the control system that guides chasing behaviour and the control system that mediates optomotor course stabilisation. Using naturalistic visual stimuli I investigated in electrophysiological experiments whether and how a prominent male-specific visual interneuron processes visual parameters of the pursued target that have been concluded in behavioural experiments to be relevant input parameters for the chasing system.

My investigations are based on behavioural experiments that were carried out with freely flying male blowflies. To reduce the complexity of the chasing flights and to facilitate the conclusions concerning relevant flight parameters, I provided to the males an artificial 'dummy target' instead of a real fly. This dummy target, a fly-sized black sphere, moved on a defined trajectory with a constant velocity. This dummy target was attractive for chasing males. The flights were recorded with two high-speed digital video cameras that were adjusted in orthogonal orientations such that it was possible to reconstruct on the basis of the simultaneously recorded 2D-video images relevant parameters of the flight trajectories in 3D. The orientation and the 3D-position of the fly as well as the 3D-position of the target were reconstructed frame by frame. Moreover, the movements of the chased target on the retina of the chasing fly could be calculated.

The aim of my behavioural experiments was, on the one hand, to characterise the flight parameters of chases and to compare them with compensatory optomotor responses as well as with cruising flights and, on the other hand, to analyse a potential interference of the optomotor system with the chasing system that may significantly impair chasing performance. During chasing, the time-varying retinal image of the small target constitutes the input to the chasing system. During self-motion, such as during flight, large-field visual image displacements are generated that constitute the input to the optomotor system. The optomotor response, a basic behavioural response observed in many animals, is thought to play a role in course control and in stabilising gaze. When a grating is rotated around flying flies, they reduce the induced large-field retinal image displacements by compensating behavioural responses, i.e. by the optomotor response. Flies perform smooth continuous body turns during both chases and optomotor flights in contrast to cruising flights where they exhibit sequences of sharp saccadic turns of high-

velocities with straight flight sections during the intersaccadic intervals. During chases each turn of the male fly towards the target inevitably leads to retinal motion of the visual environment in the opposite direction. This global motion may be compensated by the optomotor system by a counterdirected turn thereby increasing the error angle of the target on the chasing fly's retina. This 'conflict' between the chasing system and the optomotor system may significantly deteriorate the chasing performance. I investigated the interactions of the two control systems by presenting large-field image motion at different velocities to chasing males. I analysed the detailed temporal structure of the flight trajectories with respect to several parameters, e.g. retinal error angle of the target or the fly's yaw velocity. The results reveal that optomotor stimulation has no consistent impact on the chasing performance. This result indicates that the gain of the optomotor system is reduced during chasing, which can be effected by a copy of the signal of the chasing system. This kind of interaction mechanism that may allow a robust chasing performance without significant optomotor impairment, has been described in different versions in many animals.

At several levels the visual system of male flies has specialisations that are only found in males. Within the male's brain, these specialisations converge to 12 large male-specific visual interneurons, called MLGs (Male Lobula Giant neurons) that are thought to subserve chasing behaviour. I investigated in male blowflies (*Calliphora spec.*) one prominent element of the ensemble of MLGs, the MLG1 neuron, and examined which visual cues that are provided by the target are processed by the MLG1 cell. Previous behavioural and modelling studies concluded that the target's size and position on the eye of the chasing male are relevant input variables for the chasing control system. I therefore tested whether and how these parameters are represented by the responses of the MLG1 neuron. To stimulate this neuron under as naturalistic conditions as possible, I employed the reconstructed movements of the chased target on the fly's eyes as they are experienced by male flies during a real chase. These naturalistic image sequences were replayed on a monitor screen to the male blowfly while carrying out *in vivo* intracellular electrophysiological recordings of the MLG1 neuron. The MLG1 neuron shows a distinct direction selectivity, which varies within the receptive field. Large neuronal depolarisations are evoked by upward motion (preferred direction). A coherence analysis of the neuronal responses to naturalistic image sequences reveals that the target's size and position jointly influence the MLG1 responses. Thus, the hypothesis of an explicit representation of just one of these parameters within the responses of MLG1 could not be confirmed. The preferred stimulus conditions include the combined variation of retinal size and position of the target, as well as the direction, velocity and duration of the target motion within the receptive field of the MLG1 neuron. These results suggest that MLG1 plays a role in processing visual information in a chasing pathway without exclusively encoding either target size or position. Hence, I conclude that the chasing control system may employ the whole ensemble of male specific neurons for signalling the behaviourally relevant target information during

chasing. Thereby, distinct neurons could play different roles in encoding the different combinations of the target's parameters.

2 General introduction and discussion of the scientific context of fly chasing behaviour

Animals are thought to be highly adapted to their particular environmental circumstances. Also behaviour such as, for instance, feeding, mating, predation or predator avoidance, needs to be adjusted to particular tasks and environmental situations. In recent years the physiological aspect of behaviour became increasingly a focus of research, with its emphasis on the explanation of behaviour in terms of the activity of the nervous system (McFarland, 1985). Because of the relative ease with which their nervous systems can be examined electrophysiologically and by using imaging techniques, insects are well suited to study aspects of behaviour and the underlying mechanisms (e.g. Atkins, 1980; Borst and Haag, 2002; Egelhaaf and Warzecha, 1999). The speed with which especially flying insects respond to environmental stimuli necessitates specialisations at the behavioural and physiological level. In particular, the chasing behaviour of male flies, which belongs to the fastest visually controlled behaviours that can be found in nature, demands very fast neuronal processing. In the context of mating, only males of several fly species chase and catch a female conspecific (Land and Collett, 1974; Wagner 1986b). The visual system of male flies shows structural and physiological specialisations that are thought to play a role in processing the visual information relevant to mediate chasing behaviour. Most obviously, males have enlarged eyes compared to females due to a supplemental dorsofrontal eye region called 'acute zone' (Gilbert and Strausfeld, 1991; Strausfeld, 1991) where the pursued target is fixated during chasing (Boeddeker et al., 2003; Collett, 1980a; Land, 1993a; Land and Collett, 1974; Wagner, 1986b; Wehrhahn, 1979; Zeil, 1983). Furthermore, male-specific neuronal structures that are thought to mediate visual processing during chasing behaviour are found on several levels of the visual system. These physiological specialisations reach from fast-processing photoreceptors to male-specific visual interneurons that terminate on distinct neurons descending from the output regions of the visual system to the motor control centres in the thoracic ganglia (Burton and Laughlin, 2003; Francescini et al., 1981; Gronenberg and Strausfeld, 1991; Hornstein et al., 2000).

In my thesis, male blowflies (*Lucilia sp.*) were used as experimental animals to investigate chasing behaviour on the behavioural as well as on the neuronal level. In a first comprehensive main series of investigations (see chapter '3'), the chasing behaviour and its underlying control system was characterised by reconstructing the flight trajectories and body orientations of male flies chasing an artificial target moving on a circular path. For its characterisation, chasing behaviour was studied in a stationary environment. Moreover, I presented a large-field motion stimulus that constitutes a powerful stimulus for the fly's optomotor system. The optomotor

response is a reflexive turning of the animal that stabilizes the flight path by evaluating global retinal image motion (e.g. Kern and Egelhaaf, 2000). Furthermore, chasing behaviour will be compared to cruising behaviour, which is the spontaneous flight behaviour without any obvious goal. In usual environments the chasing control system might be in conflict with the optomotor system, because when chasing a target, such as a conspecific, each turn towards the target induces global retinal motion in the opposite direction. This retinal motion may lead to a compensating turning response by the optomotor system. To elucidate a potential impact of the optomotor system on chasing behaviour, the chasing performance was examined while the fly was exposed to optomotor stimulation. This examination was done by analysing the detailed time structure of the flight trajectories of the chasing flights.

The fly visual system is a well established system to study principles of visual motion processing (e.g. Krapp et al., 1998; Egelhaaf et al., 2002) and offers the opportunity to interpret electrophysiological data in a behavioural context (e.g. Kern et al., 2005). In the lobula, which is the third visual neuropil of the fly's brain, an ensemble of 12 integrating male-specific visual interneurons called MLGs (Male Lobula Giant neurons) have been characterised (Strausfeld, 1991). These MLGs have been morphologically and physiologically characterised as male-specific neurons that subserve the dorsofrontal eye region where the pursued target is fixated during chasing (Gilbert and Strausfeld, 1991; Strausfeld, 1991). In a second line of research (see chapter '4'), I investigated the response properties of the MLG1 neuron, which is one of this ensemble of male-specific visual interneurons. By *in vivo* intracellular recordings while replaying optical stimuli that simulate the visual signals received by a male fly during chasing manoeuvres, it was investigated whether the MLG1-neuron specifically represents any of the visual parameters that are thought to represent relevant input parameters to the chasing control system (Boeddeker et al., 2003).

This introductory chapter will be subdivided – along the two main lines of my experimental investigations – into two major sections, one dealing mainly with the behavioural level of chasing behaviour and the other mainly with the underlying neuronal substrate.

2.1 Chasing behaviour and optomotor response in free-flying male blowflies: Characterisation of the flight performance and interaction of the underlying control systems.

In the following I will illustrate the characteristics of chasing behaviour of a small visual target and the control system underlying this male-specific behaviour. Moreover, I will describe the characteristics of the optomotor system that is

sensitive to large-field visual motion stimuli and is presumed to exist in both sexes of flies. The potential conflict between the two visually driven following systems is not a fly-specific problem, but may be encountered by virtually all moving animals (Crapse and Sommer, 2008). The visuomotor control systems in flies are in some aspects analogous to the control systems that guide eye movements in primates. Thus, my results on the control system that guide visual pursuit of small targets and the control system that mediates following responses to large-field motion stimuli will be discussed not only in the context of studies done on other insects but also in the context of vertebrate eye movements. On the other hand, I will discuss previous results in diverse species concerning a potential interference between pursuit control systems and the optomotor controller. The literature concerned with this issue will be discussed with reference to the results of my study obtained on male flies.

2.1.1 CHASING BEHAVIOUR

Several studies performed in the last decades intended to reach an understanding of the mechanisms that underlie the chasing behaviour of male flies. Males of several fly species (Calliphoridae, Muscidae, Bibionidae, Dolichopodidae, Syrphidae) pursue potential mates or rivals in high-speed aerobatic chases (Collett, 1980a; Land, 1993a; Land, 1993b; Wagner, 1986a; Wagner, 1986b; Wagner, 1986c; Wehrhahn, 1979; Wehrhahn et al., 1982; Zeil, 1983). The chases are usually brief – they last for only up to 2s (Boeddeker et al., 2003; Collett and Land, 1975; Land and Collett, 1974). Even complicated and virtuosic flight manoeuvres of the pursued fly with sudden changes in flight direction are followed by the chasing male (Fig. 2.1). These fast and, in particular, partially unpredictable movements of flies are the reason why humans are scarcely capable to follow these animals with their eyes, which will be discussed below.

The chasing behaviour of freely flying males was analysed with the aid of video recordings (Boeddeker et al., 2003; Land and Collett, 1974; Wehrhahn, 1979; Wehrhahn et al., 1982; Wagner, 1986b; Wagner, 1986c). To catch a potential mate the male fly has to detect the target and to follow it by adjusting its speed and direction of locomotion to the course of the target. The analysis of the behaviour revealed that chasing is initiated at a distance of about 0.15m (*Fannia*) or 0.24m (*Musca*) of a conspecific flying by (Land and Collett, 1974; Wehrhahn, 1979; Wagner, 1986b). During the chase the target is fixated within the frontal part of the visual field by generating body turns towards the target (Boeddeker et al., 2003; Collett, 1980a; Land, 1993a; Land and Collett, 1975; Wagner, 1986b; Wehrhahn, 1979; Zeil, 1983). Eventually, if successful, the chase will culminate in catching the target, and – if the target turns out to be a female conspecific – in copulation (Wehrhahn, 1979; Wagner, 1986a; Wagner, 1986b).

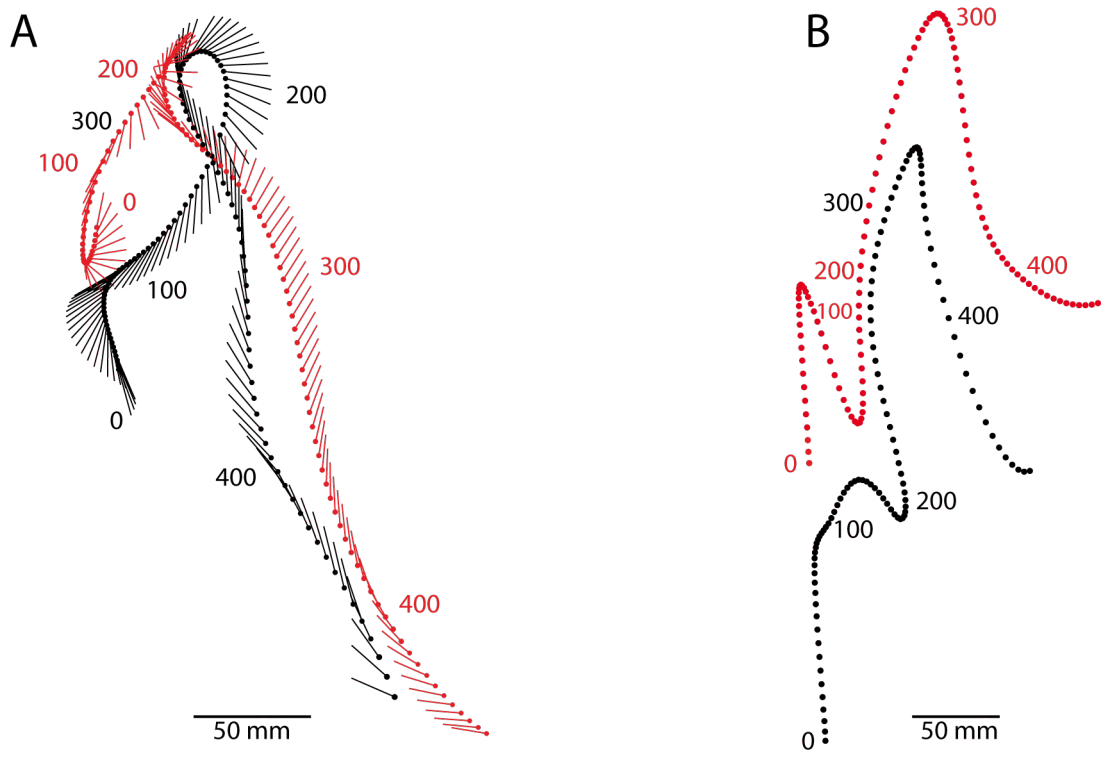

Figure 2.1 Flight trajectories of two male blowflies chasing each other, seen from below **(A)** and from the side **(B)**. The chasing fly (*black*) pursues another male blowfly (*red*) that performs virtuosic flight manoeuvres. The data of both flies are plotted every 4 ms. The position of the flies is depicted by a *dot*, the orientation of their body long axis is depicted by a *line*. The *numbers* denote the time (in ms) with respect to the start of the flight trajectories. Because the fly's pitch angle could not be determined for the entire flight (see chapter 3.2), only the fly's position is depicted in (B).

Smooth or saccadic chasing?

Previous studies on different fly species led to partly controversial conclusions with respect to the smoothness of chasing behaviour. The smoothness of a flight trajectory is determined by the velocity with which flies execute body turns around the yaw axis. Minor body rotations at low yaw velocities account for a smooth flight path, whereas fast body turns at higher yaw velocities cause a 'jerky' flight. On the one hand, in male *Fannia* (Land and Collett, 1974) and *Syritta* (Collett, 1980a) as well as in the dolichopodid fly *Poecilobothrus* (Land, 1993b) chasing has been proposed to be smooth in nature. On the other hand, male *Musca* are described to use a saccadic chasing strategy, i.e. by flying on a relatively straight course that is interrupted by fast sharp body turns, so-called saccades (Wagner, 1986b). In my study, male *Lucilia* use smooth movements to keep the target fixated in the frontal part of the retina. However, saccade-like turns occur from time to time. For instance, at the beginning of a chase an initial rapid turn serves to bring the target into the frontal part of the visual field (Fig. 2.2). Depending on the initial orientation of the fly relative to the target, this initial turn may reach high angular velocities as

are typical for saccades. Similarly, at the end of a chase the pursuer generates a final turn towards the target to grab it with his legs. Although it cannot be excluded on the basis of the present behavioural data that the chasing control system is more complex, it can be concluded that the control system guiding chasing behaviour in blowflies is smooth in nature but may occasionally be interspersed with saccades. This conclusion is supported by recent modelling studies as discussed below.

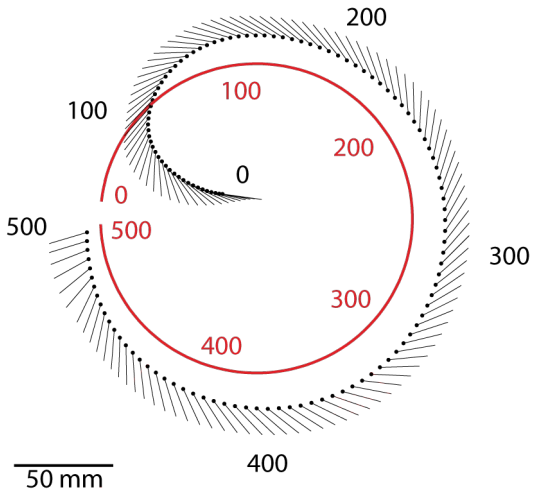

Figure 2.2 Flight trajectory of a male blowfly chasing a target, as seen from below. The fly (*black*) chases the target (*red*) which moved on a circular track. At the beginning of the chase the fly executes a large turn to bring the target into the frontal visual field. At the end of the chase the pursuer generates a turn towards the target to catch it with his legs. The target is caught after about 500ms. Data of fly and target are plotted every 4 ms. The fly is indicated by the position of its body centre (*dot*) and the orientation of the body length axis (*line*). The *numbers* denote time (in ms) with respect to the start of the trajectory.

Modelling the chasing control system

What are the mechanisms that underlie the guidance of chasing behaviour? Control theory denotes that each behavioural system is controlled by internal and environmental stimuli (McFarland, 1971). Experimental examinations about which internal and external stimuli may affect the animals' behaviour and physiology can be complemented by the use of models. These models can be simple functional schemata, constructed in order to illustrate distinct causal relationships (von Holst and Mittelstaedt, 1950) or can be more complex allowing for predictions of behaviour in quantitative terms.

The question about how the control system that guides chasing behaviour is organised was investigated in several previous studies (e.g. Boeddeker and Egelhaaf, 2005; Boeddeker et al., 2003; Collett, 1980a; Wagner, 1986b). These studies also tried to elucidate relevant input parameters of the chasing control system. Classically, chasing was described to be controlled via a feedback system, in which the error

angle of the target on the pursuer's retina plays an important role (Land and Collett, 1974). The retinal error angle is the 'target's fixation error', i.e. the deviation of the target from the frontal midline of the chasing male fly. Recent studies led to a phenomenological model of the chasing control system of male blowflies (*Lucilia*). This model is based on behavioural experiments that identified the visual cues which are used by male blowflies to guide chasing behaviour. On the one hand, the retinal size of the target controls the forward velocity of the chasing male (Boeddeker et al., 2003). Thus, for a given retinal target size the fly's forward speed is kept constant. On the other hand, the retinal position of the target (i.e. the error angle) is assumed to be a relevant input variable. By eliciting a turning response towards the target the error angle controls the fly's yaw rotation (Boeddeker et al., 2003). The turning response of the chasing male fly increases with increasing error angle, and a small error angle indicates that the pursuer keeps the target fixated frontally. The control of yaw rotation as described in male *Lucilia* is similar to that described for other male fly species (Land and Collett, 1974; Collett and Land, 1975; Srinivasan and Bernard, 1977; Wehrhahn et al., 1982; Poggio and Reichardt, 1981; Wagner, 1986b*;* Land, 1993b).

As mentioned previously, chasing behaviour in blowflies was found in the present study to be smooth in nature with occasional saccades interspersed. These results are in full accordance with results of the phenomenological model of the chasing controller (Boeddeker and Egelhaaf, 2003; Boeddeker and Egelhaaf, 2005). These modelling studies revealed that in male blowflies both types of pursuit responses (smooth and saccadic) can be accounted for by a single smooth chasing control system (Boeddeker and Egelhaaf, 2005). The smooth controller may occasionally generate saccade-like turns, for instance, when a large error angle occurs under circumstances where the target is displaced too rapidly on the pursuing fly's retina to allow the chasing male to follow it with smooth body turns, e.g. at the begin of a chase. To fixate the target in the pursuer's frontal eye region, this large error angle is converted - even by the smooth control system - into a large rapid body turn towards the target ('catch-up saccade'). Thus, saccade-like changes of body orientation in blowflies occur without the need of an extra saccade generating mechanism and can be seen as an emergent property of a smooth chasing system.

2.1.2 VISUAL PURSUIT OF SMALL TARGETS IN INSECTS AND PRIMATES

A control system that guides visual pursuit of small targets is not only a characteristic of male flies. Pursuit of small targets has been described in different insects as well as in primates, including humans. Insects may use target pursuit for catching female conspecifics, such as male Dipteran flies (see above) or drone bees (Gries and Koeniger, 1996). In the context of mating behaviour drone bees chase the queen and fixate their target in a specific eye region, which is the dorsofrontal part of the visual field (van Praagh et al., 1980). Other insects may use target pursuit in

the context of feeding behaviour, and this behaviour is, of course, common to both sexes. For instance, praying mantids sit in ambush and detect their potential prey by using small-field visual cues (Rossel, 1980). Fast-flying dragonflies pursue other insects for predation (Olberg et al., 2000). Primates including humans are among the few vertebrate species that are able to track small moving targets by means of eye movements (reviews: Ilg, 1997; Land, 1992; Land, 1999).

To achieve visual pursuit, different species may need to move different parts of their body: Because vertebrates possess mobile eyes, visual pursuit may solely be effected by eye-movements. Only large deviations of retinal target position from the fovea may be supported by additional movements of the head and the body. By contrast, in insects the eyes are fixed within the head. Therefore, visual target pursuits are performed by head movements and may include movements of the entire body.

Primates and some insects are known to use two types of strategies for pursuit: the smooth and the saccadic pursuit strategy. For instance, in praying mantids both types of pursuit strategies can be clearly distinguished. When sitting in ambush, the praying mantis initially fixates a target by rapid, saccade-like head and body movements. After being fixated, moving targets are held in the fovea either by smooth or by saccadic pursuit eye (i.e. head) movements. The degree to which either tracking strategy is employed depends on the features of the background, but also on the velocity of the target (Rossel, 1980).

Pursuit eye movements in primates

Among vertebrates, pursuit eye movements induced by small targets are best investigated in primates including humans (reviews: Ilg, 1997; Land, 1992; Land, 1999; Lisberger et al., 1987). Eye movements during target pursuit are smooth and cannot be performed at will. They are elicited by the appearance of a moving target in the visual field. These slow (<50°/s) eye movements stabilize the projection of the moving target within the fovea and correct for any velocity error between eye and target (Meyer et al., 1985). Smooth pursuit eye movements are elicited with a latency of around 100 ms (Steinbach, 1976; Braun et al., 2006). Because of this inherent delay, large position errors may arise during visual pursuit of a target that changes its direction abruptly. Moreover, smooth eye movements cannot reach large velocities in a short period of time and cannot lead to fixation of very fast targets (Fig. 2.3A), which results in an accumulation of position error. To avoid the build up of position error during target pursuit, the oculomotor system has developed two strategies:

First, smooth pursuit can be combined with saccades. These very fast (50-1000°/s) eye movements correct for a position error between eyes and target (Meyer et al., 1985). When the eye is lagging behind the target during pursuit, the execution of a saccade helps catching up with the target (hence their name: catch-up saccades, Fig. 2.3B) (de Brouwer et al., 2002). The saccade thus greatly improves the tracking of a

target that changes its direction rapidly. With respect to the employed mechanisms – smooth pursuit combined with catch-up saccades – target pursuit in primates is very similar to target chasing in male flies (reviews: Ilg, 1997; Land, 1992; Land, 1999). By the way, saccades are not only used in combination with pursuit; basically, we generate several times in a second saccadic gaze shifts in stationary surroundings, for instance during reading or inspecting a picture (review: Kennedy et al., 2000). These saccadic eye movements relocate the images of the world on the retina. Between saccades, in periods of nearly stationary viewing, the eyes fixate an image that may prove to be of particular interest (Land et al., 1999).

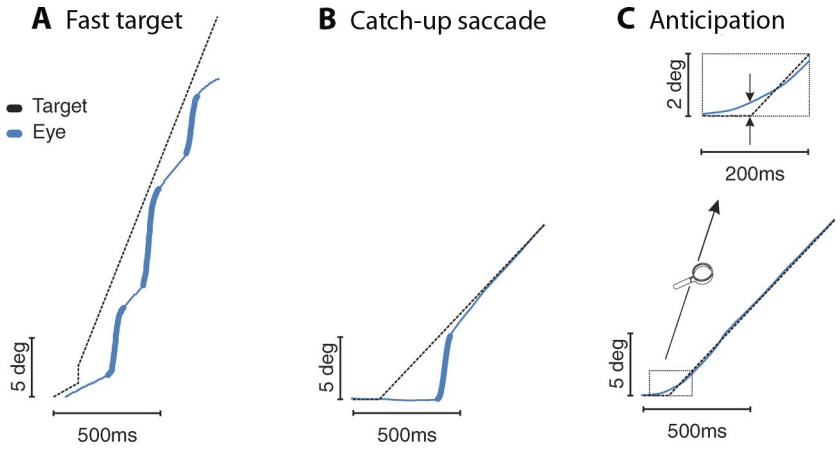

Figure 2.3. Oculomotor strategies in visual tracking of primates (Orban de Xivry and Lefèvre, 2007). For each panel, eye and target position are represented *versus* time. **(A)** The target (*black*) moving at 50°/s is followed by smooth pursuit eye movements (*thin sections of blue trace*). The eye velocity does not match target velocity. Therefore, catch-up saccades are triggered (*thick sections of blue trace*) to bring back the target into the fovea and to continue with smooth pursuit. **(B)** Initially, the target is stationary and then starts moving with a velocity of 18°/s. Due to its inherent delay, the oculomotor system lags behind the target. Around 200ms after target motion onset, a catch-up saccade (*thick section of blue trace*) is executed to cancel the position error. Thereafter the moving target is followed accurately by smooth pursuit. **(C)** After presenting a fixation point, a target moving at constant speed (18°/s) in a constant direction was presented repeatedly with the same time delay between fixation point disappearance and target appearance. When targets move predictably, smooth pursuit eye movements are initiated in anticipation of a moving target. The eyes (*blue trace*) begin to move before the target (*black trace*) does. The oculomotor system predicts the time of target motion onset and the pursuit eye movements are scaled to the velocity of the expected target velocity. The inset provides a zoom around target motion onset. The arrows highlight the advance in position of the eye with respect to the target at its onset.

Second, another strategy of the oculomotor system to avoid position errors during target pursuit is the use of predictions to anticipate the future target trajectory (Fig. 2.3C; Bahill and McDonald, 1983; Barnes and Asselman, 1991, Freyberg and Ilg, 2008). However, this strategy is limited to conditions where the target trajectory is predictable. It fails in situations with unpredictably moving targets with sudden

changes in direction, as may be the case when we try to visually follow the flight trajectory of a chasing male blowfly. Mainly due to its inherent delay (see above), the oculomotor system is too slow to pursue accurately a fast, erratically moving target. Even when the target velocity is low, such as a flying mosquito, its jerky flight trajectory makes it very difficult to catch the harasser, which is a good example of a very frustrating situation, especially in the middle of a short night.

Visual input to pursuit eye movements in vertebrates

What visual cues of the target are used as input by the pursuit control systems? Throughout the last decades, neuroscientists considered the target velocity to be the input of the smooth pursuit system, and the target position to be the input of the saccade controller. The retinal inputs subserving the oculomotor system were classically thought to segregate into two parallel cortical pathways, controlling mainly the saccadic and the smooth pursuit system, respectively (Tian and Lynch, 1996; Rosano et al., 2002). However, recent experiments revealed that the saccadic and smooth pursuit systems share the same inputs, i.e. the position and motion signals. On the one hand, experiments in cats revealed the first evidence of a contribution of the target position to the smooth pursuit system, which is consistent with modelling studies (Lefèvre et al., 1994; Missal et al., 2002). Furthermore, behavioural experiments in primates provided evidence for a position input to the smooth pursuit system: During ongoing target pursuit, smooth eye movements were elicited towards a second target that was flashed aside the pursuit path (Blohm et al., 2005). On the other hand, although saccades towards stationary targets typically correct for the position error between eye and target, the amplitude of accurate catch-up saccades is not related to the position error alone but also to target velocity (Kim et al., 1997). If this were not the case, saccades directed towards a moving target would always fall short because of inevitable latencies of the behavioural response. Therefore, in programming the saccade amplitude, the motion of the target must be taken into account (de Brower et al., 2001).

2.1.3 FOLLOWING RESPONSES TO LARGE-FIELD MOTION STIMULI IN INSECTS AND VERTEBRATES

All above mentioned examples are concerned with the visual pursuit of relatively small targets. Visual following responses emerge as well in response to motion of large-field patterns, such as the motion of the entire visual field (global motion) as is induced, for instance, during self-motion in a structured environment. These following responses to moving visual large-field stimuli are often called optomotor response, which is one of the basic behavioural responses observed in many animals. The optomotor response is known in many invertebrates, and it is also known as optokinetic nystagmus (OKN) in vertebrates including humans.

The optomotor system has been well studied in flies in tethered flight (reviews: Egelhaaf and Borst, 1993; Hengstenberg, 1993; Heisenberg and Wolf, 1984; Reichardt, 1993) as well as in free flight (Collett, 1980a; Collett, 1980b; Duistermars et al., 2007; Frye and Dickinson, 2007; Land and Collett, 1974; Tammero and Dickinson, 2002; Wagner, 1986a; Wagner, 1986b; Wagner, 1986c; Götz, 1975). By compensatory reactions mediated by head or body movements the optomotor system is thought to try to balance out the image velocity the animal observes on both sides during rotation (Fig. 2.4). These optomotor responses are thought to play a role to stabilize gaze and to correct for deviations from the intended path of locomotion (reviews: Collett et al., 1993; Kern and Egelhaaf, 2000; Strauss and Heisenberg, 1990; Wehner, 1981).

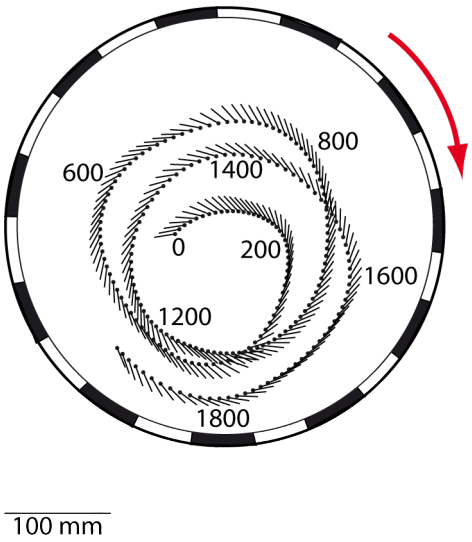

Figure 2.4 Flight trajectory of an optomotor flight, as seen from below. The male blowfly that flies in a cylindrical flight arena is confronted with a large-field moving stimulus, which is a vertical grating rotating around the animal. The motion direction of this *grating* (background) is indicated by the *red arrow*. The fly tends to compensate this rotation by following the background motion by a combination of body turns and translational movements. The background motion velocity is 365°/s. The data of the fly are plotted every 8 ms. Same plotting conventions as in Figure 2.2.

Optomotor course control as well as the visual stabilisation of gaze by compensatory eye movements is also of importance in vertebrates (Miles and Wallmann, 1993). The so-called optokinetic nystagmus (OKN) generates eye movements that are performed reflexively if large parts of the environment move coherently across the retina (reviews: e.g. Ilg, 1997; Miles, 1993). The OKN can be understood as a negative feedback system that minimizes the retinal slip of the visual image by eye movements (Miles and Wallman, 1993). During self-motion, for instance, while we sit in a moving train and look out of the window, the eyes inevitably follow the moving landscape. Thereby, the eye movements change between slow and fast

phases. The slow phases of the OKN correspond to smooth eye movements that follow the moving large-field stimulus. The quick phases of the OKN have a similar velocity as visually guided saccades. These fast movements move the eyes back in the orbit to allow them to follow the large-field motion stimulus again (Ilg, 1997).

2.1.4 INTERACTION BETWEEN THE TWO CONTROL SYSTEMS GUIDING THE VISUAL PURSUIT OF SMALL TARGETS AND THE FOLLOWING OF LARGE-FIELD MOTION

Above I described in insects and primates small-field sensitive pursuit systems as well as following responses to large-field motion. The two control systems that guide the small-field and large-field following responses may fulfil their respective behavioural task accurately when working in isolation. However, there may be situations in which both systems may interfere with each other. For instance, let us assume a male blowfly chasing a conspecific and thereby making intentional turns towards its target. These target-induced turns into a given direction in front of a structured background inevitably lead to global motion in the opposite direction on the fly's retina. This large-field motion may activate the optomotor system. Thus, during chasing in a natural environment the two visually driven following systems may be active simultaneously. In 'compensating' for the global image motion, the optomotor system may evoke a counterdirected turning response. This optomotor response would be opposite to the intended turn towards the target mediated by the chasing system. As a consequence, the optomotor response may corrupt the success of the intended behaviour of the chasing fly.

This interference of two potentially conflicting control systems is not restricted to flies. In contrast, this is a general problem of many moving animals. For instance, a similar situation is given in humans during pursuit eye movements in a textured environment that inevitably induce global motion of the visual field in the opposite direction. This large-field motion may activate the OKN that may generate compensating eye movements.

Interaction of the chasing and optomotor controllers in male blowflies

I investigated the issue whether optomotor stimulation has an impact on the chasing performance of male blowflies. To find out whether these two components of visuo-motor behaviour interfere with each other my experiments were designed to stimulate the two control systems simultaneously. Male blowflies chasing a target in a flight arena were confronted with a vertical grating pattern positioned around the flight arena. This visual environment could be moved at different velocities. In the different experiments, the chasing males were confronted with a stationary background, or with slow or fast background motion moving in the same or opposite direction as the target, respectively. Thus, the optomotor stimulation was decreased

or increased with respect to the normal stationary environment and to the direction of target motion.

Large background velocities reduced the catching success to some extent. However, when counting the number of flies that were actually flying, I found a significant decrease in flight frequency during fast background motion. These results suggest that the catching success does not deteriorate as a consequence of background motion after the flies initiate a chase, if it is normalised to flight activity. Since optomotor stimulation alone strongly affects the fly's yaw- and forward velocity (my study; see also Collett, 1980a; Collett, 1980b), one might expect that a chasing fly could be somewhat retarded by decreased, and accelerated by increased large-field velocity. However, no such effect could be found during chases for the flight parameters yaw velocity, forward velocity, turning frequency, the fly's distance to the target and retinal target velocity. Furthermore, the retinal error angle of the target was analysed. During chasing, the retinal image of the target was found to reside within the frontal region of the retina, which is in accordance with previous studies (e.g. Boeddeker et al 2003; Collett, 1980a; Land, 1993a; Land, 1993b; Land and Collett, 1974; Wagner, 1986b; Wehrhahn 1979; Zeil 1983). Altogether, my results indicate that optomotor stimuli have no consistent impact on the fine structure of chasing behaviour. This indicates that the optomotor system is not significantly impeding the performance of chasing behaviour. Therefore, it might not affect the success (i.e. capture) or failure (no capture) of chases.

Mechanisms of interaction between the two control systems

Which mechanism may explain the robust chasing performance during optomotor stimulation in blowflies? The interaction between the chasing and the optomotor controllers may be subject to some kind of gain control. This could be a gain reduction of the optomotor system during chasing behaviour. How might such a gain reduction be accomplished? The presence of an 'appropriate' visual input stimulus (i.e. the error angle of a small target) may activate the chasing system which then generates an output signal (i.e. the turning command). A copy of this chasing signal may reduce the gain of the optomotor system (Fig. 2.5). Despite the optomotor system may receive a visual input stimulus (i.e. large-field motion) while chasing a moving target, its decreased gain may not allow the generation of a large optomotor response. Thus, the chasing system dominates the overall turning behaviour. This gain control may prevent compensatory optomotor responses during intended turns towards a chased target. Due to the smoothness of chasing behaviour, a copy of the chasing signal possibly as well inhibits the generation of saccades during chasing. Sequences of rapid saccadic yaw turns are typically exhibited by flies that perform cruising flights, which are flights without any obvious goal (Fig. 2.6) (Bender and Dickinson, 2006; Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999; Wagner, 1986c).

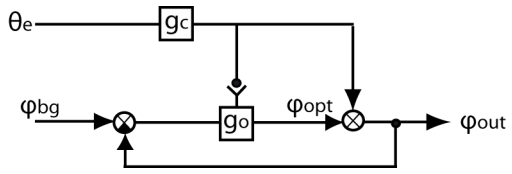

Figure 2.5 Cybernetic model of a possible interaction between the chasing and the optomotor system of flies. The input to the chasing system is *θe*, i.e. the retinal error angle of the target. *gc* and *go* are the internal gains of the chasing system and the optomotor system, respectively. A copy of the chasing signal reduces the gain of the optomotor system. The input to the optomotor system is provided by *φbg* that is the angular velocity of the environment (i.e. background). *φopt* is the output of the optomotor system, and *φout* is the final behavioural (turning) response.

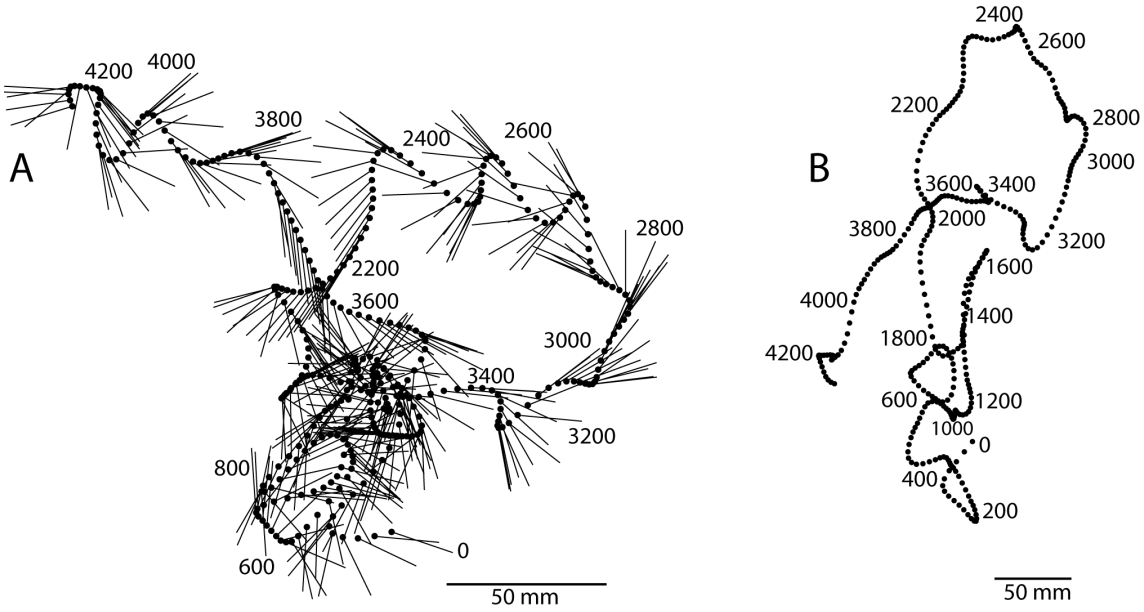

Figure 2.6 Flight trajectory of a male blowfly performing a cruising flight, as seen from below **(A)** and from the side **(B)**. **(A)** A fly performs the saccadic flight strategy that is typical for cruising flights. The flies execute sequences of brief but rapid saccadic turns in both directions with high angular velocities. Between the saccades, there are longer translational segments of straight flight, i.e. the fly performs little or no rotation. The fly is indicated by the position of its body centre (*dot*) and the orientation of the body length axis (*line*). At the begin of the flight, the fly mainly moves in the vertical directions, which can be seen in a side view of the flight in **(B)**. Because the fly's pitch angle could not be determined for the entire flight (see chapter 3.2), only the fly's position is depicted in (B). Data of the fly are plotted every 16 ms. *Numbers* denote time (in ms) with respect to the start of the trajectory.

Where in the visual pathway does the copy of the chasing signal interact with the optomotor system? Previous studies indicate that the motion signals carried by the optomotor system are likely to be low-pass filtered (Egelhaaf, 1987; Warzecha and Egelhaaf, 1996; Wolf and Heisenberg, 1990). This filtering is supposed to be accomplished between the third visual neuropil, the lobula plate that contains the

neuronal substrate subserving the input to the optomotor system (see below), and the final behavioural optomotor response. The time constant of the optomotor system's low-pass filter was supposed to be in the range of several hundred ms (about 750 ms) (Warzecha and Egelhaaf, 1996). The level at which the chasing signal attains the optomotor pathway is significant for the chasing performance: If the chasing signal would target at 'early' levels within the optomotor pathway before the low-pass filtering takes place, this signal would be subject to the same large time constant. This, in turn, would have the consequence that the gain reduction of optomotor response were delayed with respect to the chasing signal and thus would partially interfere with chasing performance. Therefore, it is likely that the chasing signal targets the optomotor gain at a 'higher' level within the optomotor pathway, i.e. closer to the motor output than to the lobula plate.

The optomotor gain reduction during chasing may denote a good adaptation to natural chasing situations: During the brief (0.5-2s) chase (Boeddeker et al., 2003; Collett and Land, 1975; Land and Collett, 1974), the pursuer may 'pay full attention' to the target and 'ignore' the background. That may not be a dangerous strategy while following the flight path of the conspecific, since then the chasing male may have no risk to bump into potential obstacles. A previous study proposed for male hoverflies (*Syritta*) that a similar mechanism may account for the interaction between the chasing and the optomotor controllers, in combination with some kind of additive interaction: Both control systems were revealed to combine additively, but to give the required chasing performance, the gain of the chasing system was concluded to be larger than that of the optomotor system (Collett, 1980a).

Due to the experimental setup as used in my study, the dummy target that was chased by the male flies moved at a constant angular velocity. This indicates that low-frequency visual input is present during target chasing. Since the target was found to be well fixated within the frontal eye region, the chasing control system most likely responds to this low-frequency input. The robustness of the chasing performance during optomotor stimulation is not self-evident, since the response properties of the optomotor system are found in several fly species to be especially sensitive at low oscillation frequencies around 1-2Hz (Duistermars et al., 2007; Egelhaaf, 1987; Hausen, 1982a; Hausen, 1982b; reviews: Collett et al., 1993; Egelhaaf and Borst, 1993; Egelhaaf and Warzecha, 1999; Reichert, 1993). It is likely that the optomotor system in *Lucilia* shows similar functional characteristics.

Another possibility for the interaction of the optomotor control system and the small-field selective object fixation system (see below) was proposed to be a dynamic separation of the two control systems in terms of different temporal frequency characteristics (Collett, 1980a; Duistermars et al., 2007; Egelhaaf, 1987). Due to the fast characteristics of chasing behaviour, one would expect that the chasing system responds best to transient movements. Up to now, the temporal frequency properties of the chasing system has not been analysed yet. However, the results of

my study reveal that it is unlikely that dynamic separation of the two control systems accounts for the robust performance of chasing during optomotor stimulation.

The analysis of the frequency content of the velocity at which the target moves on the pursuer's retina shows that high frequencies of up to 30-50Hz are prominent in the fluctuations of the retinal error angle during chasing flights. These high-frequency fluctuations may be a consequence of a high gain of the chasing controller. Since all biological systems lag the input with an inherent delay, the system may lead to fluctuations if it operates at high gain in order to compensate disturbances efficiently (Boeddeker and Egelhaaf, 2003; Warzecha and Egelhaaf, 1996; review: Land, 1992).

Interaction of the optomotor controllers with other behavioural control systems in invertebrates

The problem of an interference of two control systems was examined in diverse insect species. In flies, the interaction of the optomotor system was analysed for another behavioural system which exists in both sexes: the small-field sensitive object-detection and -fixation system (Egelhaaf, 1985a, Egelhaaf, 1985b, Egelhaaf, 1985c). Flies can use motion cues, i.e. the relative motion information generated at the edges of objects, to detect these objects in front of a structured background (Reichardt et al., 1983; Egelhaaf, 1985a; Kimmerle et al., 1997). In early studies the optomotor response and object fixation behaviour in *Musca* were supposed to combine additively (Srinivasan and Bernard, 1977). Subsequent studies in *Musca* revealed that due to different temporal frequency characteristics of the object detection system and the optomotor system, the fly can fixate objects moving in their visual field by turning towards objects without both systems impeding each other (Egelhaaf, 1987; Egelhaaf et al., 1988). Recently, similar dynamic properties of these two control systems were found in the fruitfly *Drosophila* (Duistermars et al., 2007; Sherman and Dickinson, 2003).

Praying mantids locate their prey visually. Thereby, mantids pursue a moving prey in front of a homogenous background by smooth head movements. However, in front of a structured background mantids track their prey with a series of saccades (Rossel, 1980). It seems likely that mantids have not fully solved the problem of eliminating the optomotor response during target pursuit (review: Kral, 2003). Moreover, Böhm and others (Böhm et al., 1991) found that in crickets optomotor stimuli have an impact on the phonotactic orientation in an additive way, such that large-field motion additively shifted the direction of walking elicited by a calling song. Furthermore, a recent study in *Drosophila* shows that an attractive odorant increases the ability of flies to stabilize image motion. This indicates a modification of optomotor control in a context-dependent manner which enables an animal to fly straight up an odour plume and to approach odiferous objects (Chow and Frye, 2008).

Interaction between the two control systems in primates guiding the visual pursuit of small targets and the following of large-field motion

A similar situation of two potentially conflicting control systems may be given during target pursuit in primates: Smooth pursuit eye movements in response to a small moving target induce in a textured environment large-field motion in the visual field in the opposite direction. This large-field motion may represent a powerful stimulus for the OKN. This potential conflict has been the subject of many studies in primates and humans: Smooth pursuit eye movements induced by a small moving target were investigated while the background was stationary or moving. These studies reveal partially contradicting results, which might originate from different experimental designs:

Stationary textured backgrounds substantially reduced the initial eye acceleration during target pursuit (Keller and Khan, 1986; Kimming et al., 1992; Masson et al., 1995; Mohrmann and Thier, 1995; Niemann and Hoffmann, 1997; Spering and Gegenfurtner, 2007; Yee et al., 1983). As a consequence, saccades were generated more frequently (Collewijn and Tamminga, 1984). A textured background moving in the opposite direction to the target impaired initial eye acceleration of target pursuit (Keller and Khan, 1986; Masson et al., 1995; Yee et al., 1983), or enlarged the initial eye acceleration (Niemann and Hoffmann, 1997), or had no effects on the target pursuit performance (Schwarz and Ilg, 1999). A background moving in the same direction as the target improved target pursuit (Masson et al., 1995; Yee et al., 1983) or led to a marked transient perturbation of target pursuit (Schwarz and Ilg, 1999). Another study found that moving textured backgrounds enhanced target pursuit irrespective of the direction of background motion (Spering and Gegenfurtner, 2007). These examples show multiple influences of a background on target pursuit. However, many of these studies found an impact on eye acceleration only during the *initial* phase of target pursuit. Only few studies found that steady-state pursuit performance, measured several hundred milliseconds after the onset of pursuit, was affected only marginally (Keller and Khan, 1986; Lindner et al., 2001) or was significantly reduced by the presence of a stationary or a moving textured background (Masson et al., 1995; Mohrmann and Thier, 1995).

Nevertheless, during target pursuit, some form of reduction of the OKN-response may take place. Lindner and colleagues (Lindner et al., 2001) found that the sensitivity of OKN is strongly reduced for large-field stimuli moving in the opposite direction to the target. Some extra-retinal information may be required for the elimination of the OKN during target pursuit, since neither the eye-movement induced retinal image motion per se nor the relative motion between the pursuit target and background are sufficient for an elimination of OKN during target pursuit (Lindner and Ilg, 2006).

2.2 Characterisation of a blowfly male-specific neuron using behaviourally generated visual stimuli

So far, I scrutinised the small-field and large-field sensitive following systems in flies and primates with respect to the mechanisms that underlie the interaction between these systems and with respect to their functional relevance. Now, I will look inside the chasing system and address the following questions: What is the neuronal substrate that provides the input to the chasing system? Which visual cues of the target are processed by these neurons and which visual stimuli induce large neuronal responses? Whereas in primates, pursuit of small targets can be executed by males and females, chasing behaviour in flies is only performed by males. Therefore, the question for the neural substrate underlying chasing behaviour is particularly interesting in male flies. In the following I will try to answer these questions. I will depict male-specific specialisations that reside at several levels of the visual system and that are associated with chasing behaviour on the one hand, and present the results of investigations of one male-specific neuron that is presumed to subserve chasing behaviour in the blowfly *Lucilia* on the other hand. Moreover, the neural basis of behavioural responses to small-field moving targets has also being investigated in other insect species. Here, I will select two aerobatic insect species in which previous investigations found small-field sensitive visual neurons that are thought to process the visual information of the pursued target. Finally, the question of what neuronal substrate providing input may also arise for the fly's optomotor system that, however, is assumed to exist in both sexes. Therefore, I will conclude with a short overview of the visual neurons that are assumed to underlie the fly's optomotor response by processing large-field visual cues.

2.2.1 NEURONAL SUBSTRATE UNDERLYING CHASING BEHAVIOUR RECEIVES VISUAL INFORMATION BY THE MALE ACUTE ZONE

When a male blowfly chases a conspecific, the chasing fly continuously changes the orientation of its body long axis to keep the target fixated in a specific part of the visual field, the so-called 'acute zone' (Collett, 1980; Land, 1993a; Land and Collett, 1975; Wagner, 1986b; Wehrhahn, 1979; Zeil, 1983b). In several species of flies the acute zone is the dorso-frontal eye region that is enlarged in males compared to females (Fig. 2.7) (*Musca*: Wagner, 1986b; Wehrhahn et al., 1982; *Poecilobothrus*: Land, 1993a; *Syritta*: Collett and Land, 1975). In the male blowfly *Calliphora* the acute zone lays 20-30° above the equator and differs from other parts of the eye mainly by increased acuity (Fig. 2.8).

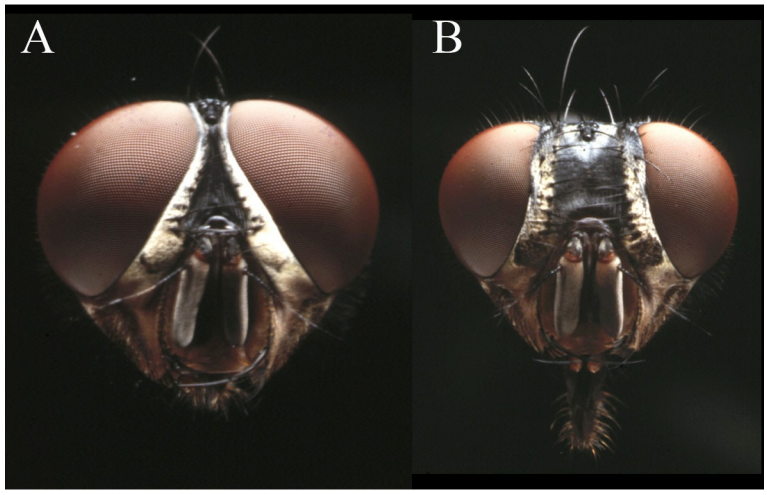

Figure 2.7 Sexual dimorphism of blowfly eyes. Views of the heads of a male **(A)** and a female **(B)** *Calliphora erythrocephala* from the front. The dorsofrontal eye region of the male is enlarged compared to that of the female. This enlarged eye region contributes to the male acute zone (pictures taken by Hans van Hateren: http://hlab.phys.rug.nl).

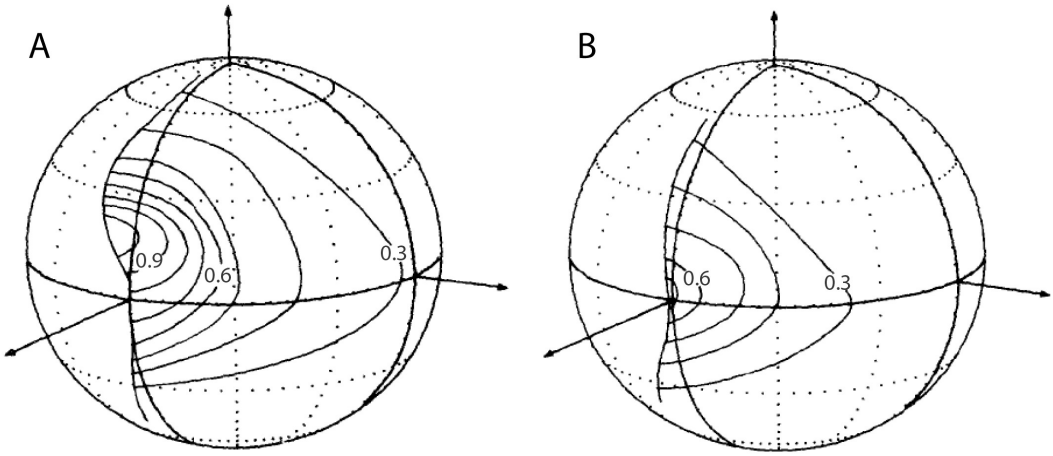

Figure 2.8 The region of increased acuity in male **(A)** and female **(B)** *Calliphora erythrocephala* (Land and Eckert, 1985). The figures show plots of the densities of ommatidial axes (axes/degree^2) on the polar coordinate system of the fly's visual field (for explanation of the polar coordinate system of the fly's visual field see Fig. 4.1). The axis density is directly related to facet area and thus is a measure for the acuity. The *numbers* represent the ratio of the axis density to the maximum density. Notice within the male acute zone the greater axis density and its more dorsal location relative to the female region of increased acuity.

Such specialised eye regions are as well characterised in other insects. Males and females of predatory species such as dragonflies and praying mantids are supposed to use the acute zone to catch their prey (mantids: Rossel, 1980; dragonflies: Frye and Olberg, 1995). Amongst non-predatory flies and bees only the males have an acute zone that suggests for the significance of this eye region in sexual pursuit (hoverflies: Nordström et al., 2006; blowflies: Land and Eckert, 1985; drone bees: van Praagh et al., 1980). Besides exceptions (see below), the information originating in the acute zone is processed in similar ways as in the other parts of the eye through the successive optic lobes representing different aspects of information processing (Fig.

2.9). Starting in the retina the photoreceptors transduce light intensities into graded changes of the photoreceptor membrane potential (e.g. Juusola et al., 1994). These signals are transmitted to the first optic lobe, the lamina, where temporal signal processing takes place (e.g. Uusitalo et al., 1995). In the second optic lobe, the medulla, visual motion is detected (e.g. Douglass and Strausfeld, 1995). So far all information is processed in separate columns with each column representing one 'pixel' of the fly's retinal image. The third optic lobe is the lobula complex which is subdivided into the lobula and the lobula plate. In the lobula plate an ensemble of about 60 direction-selective motion sensitive elements, the so called tangential cells, is very well investigated (e.g. Hausen, 1976, Hausen, 1982a; Hausen, 1982b; Hengstenberg, 1982; Egelhaaf, 1987; Borst and Haag, 2002; Egelhaaf et al., 2002).

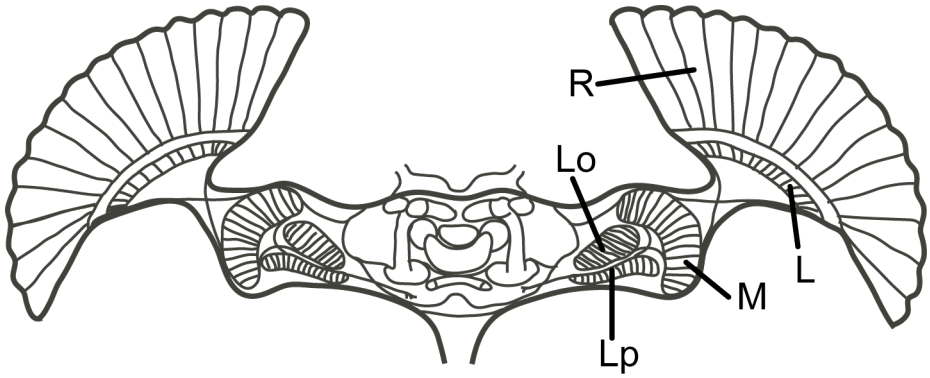

Figure 2.9 Sketch of a horizontal section through the fly visual system (modified from Hausen, 1982). *R* Retina: the lines indicate the ommatidial structure of the compound eye. *L* Lamina: the first optic lobe; information processing takes place in cartridges corresponding to the ommatidial organisation. In the second optic lobe, the medulla (*M*) the retinotopic structure is preserved. Here, motion computation is expected to take place. The axons that leave the medulla are processed to the third optic lobe, the lobula complex that consist of the lobula (*Lo*) and lobula-plate (*Lp*). In the lobula-complex large interneurons, the tangential cells, spatially integrate the retinotopic information received from the medulla.

Within acute zones, there are several specialisations that reach from the optics of the eye over the photoreceptors to the neurons that process the information from that eye region. First, two important features improve the spatial acuity, which are the size of the facet lenses and the interommatidial angle (Land, 1997). Each sampling unit of the compound eye, the ommatidium, has its own lens. Because there is a large number of lenses, they are necessarily small. As a consequence of the wave nature of light, diffraction limits the resolution of these tiny lenses. Larger facets within the acute zone (*Calliphora*: male 37 µm, female 29 µm) thus increase the quality of the optics by increased photon catch (Land and Eckert, 1985). The other structural feature that affects the performance of an eye is the interommatidial angle (Land, 1997). This angle determines the spatial acuity, because it limits the finest grating that can be resolved: When an eye views a fine grating, single stripes

will be resolved if there are two detectors that view each cycle of the grating. The interommatidial angle in the Dipteran eye is smaller in the acute zone of males than in the corresponding eye region of females (*Calliphora*: male 1.07°, female 1.28°) (Land and Eckert, 1985).

Second, whereas large parts of the eye contain several types of photoreceptors that are sensitive to different spectral sensitivities, the acute zones often contain only reduced receptor sets with similar spectral sensitivities (Francescini et al., 1981; Hardie, 1986; Stavenga, 1992). Thus, while other parts of the retina possess different colour receptors, the male acute zone is colour blind. The male acute-zone receptors do not only share the same spectral sensitivities as motion-sensitive photoreceptors; the axons of male acute-zone receptors synapse together with the axons of the other photoreceptors in the lamina (Francescini et al., 1981). By increasing the signal-to-noise ratio, this additional input to the pooling of photoreceptors in the lamina increases the sensitivity of this eye region.

Third, the retinotopic projections from the acute zone project in the dorsal region of the male lobula that contains about 12 identified male-specific tangential cells, the so-called male lobula giant cells (MLGs, Fig. 2.10). Additionally, within this dorsal region of the lobula an array of male-specific columnar neurons (MCols) have been identified (Hausen and Strausfeld, 1980; Gilbert and Strausfeld, 1991). As revealed by physiological and anatomical studies the receptive fields of 10 of the 12 MLGs as well as three types of MCols subtend the area of the acute zone (Strausfeld, 1991). The axons of most of the MLGs terminate at the same (ipsilateral) side of the brain hemisphere within the region of interneurons that descend to the motor control centres in the thorax. These descending neurons are dye-coupled in the thoracic ganglia revealing connections with motor neurons that supply the neck muscles and control the wing beat amplitude (Gronenberg and Strausfeld, 1991). The axons of two types of MCols and of MLG4 project contralaterally. Moreover, MLG1 branches, together with the contralateral MLG2, on contralateral descending neurons. The MLG1 neuron has been suggested to be dye-coupled and, thus, possibly electrically coupled with its contralateral counterpart (Gilbert and Strausfeld 1991; Wachenfeld, 1994). This coupling of heterolateral elements may be the basis of the motion sensitivity of MLG1 in both halves of the visual system.

The response properties of all MLGs are generally found to be directionally selective. Furthermore, they show a higher sensitivity to motion of small targets than to wide-field motion (Gilbert and Strausfeld, 1991). The MLG1 neuron has been investigated best so far with respect to its response properties: it exhibits pronounced direction selectivity, its receptive field subtends the area of the acute zone, and it responds well to small moving objects (Gilbert and Strausfeld, 1991; Wachenfeld, 1994). Due to their receptive field properties and response characteristics the male-specific neurons are presumed to be involved in guiding chasing behaviour.

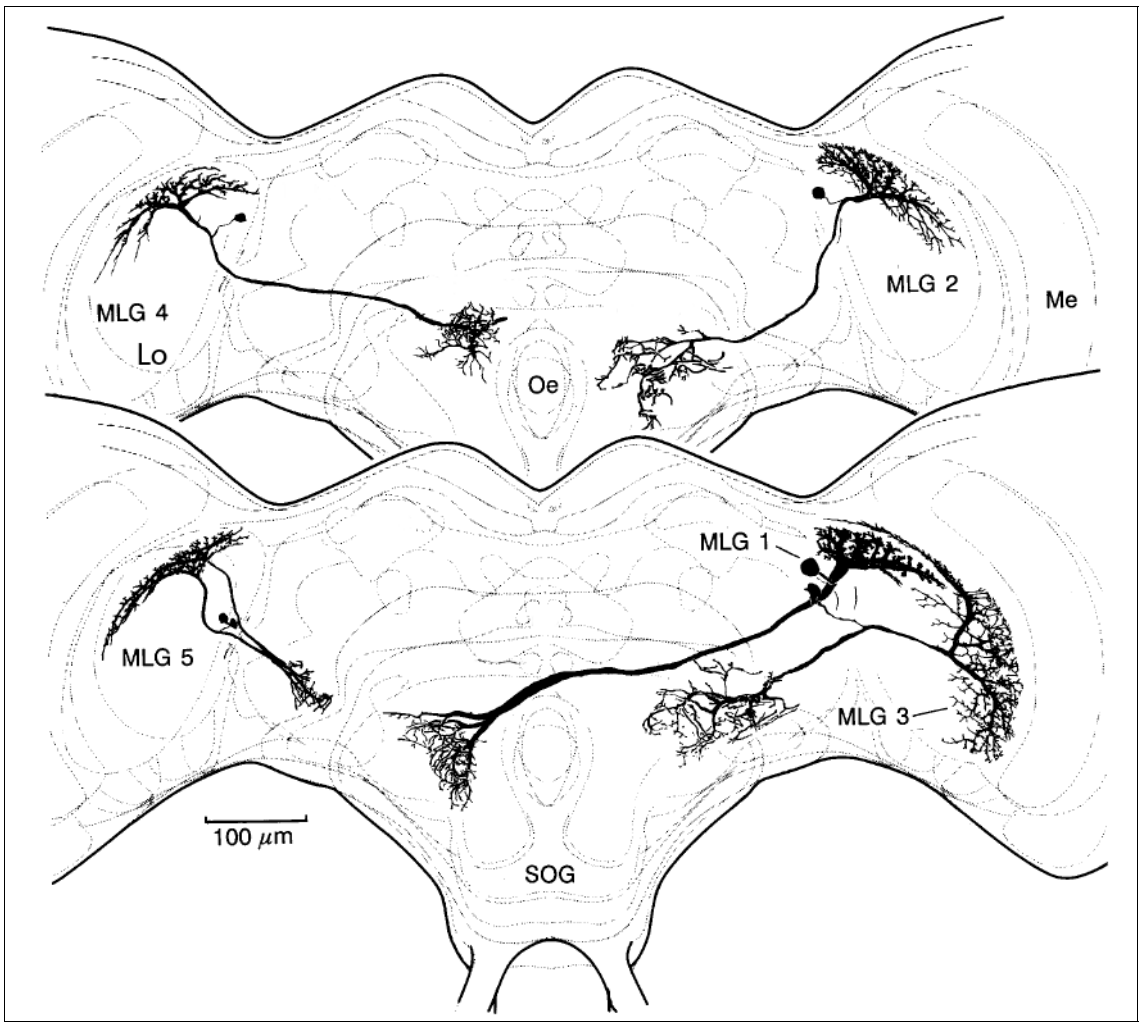

Figure 2.10 Sections of the brain of *Calliphora* showing the relative anatomical arrangement of MLG 1-5 (modified from Gilbert and Strausfeld, 1991). The dendrites of MLG1, MLG2, MLG4 and MLG5 occupy the dorsal region of the lobula (*Lo*) that has been shown to receive retinotopic projections from the acute zone. Note the massive axon and primary dendrites of the MLG1 neuron. The axon of MLG1 extends to the contralateral brain hemisphere. *Me* Medulla, *Oe* Oesophageal foramen, *SOG* Suboesophageal ganglion.

2.2.2 CHARACTERISATION OF MLG1 USING NATURALISTIC STIMULI

What visual cues of the target are represented by the MLGs? Phenomenological models of the control system underlying chasing behaviour (Boeddeker et al., 2003; Land and Collett 1974) revealed that the most important visual cues used for a chasing controller are the target position (error angle) on the retina of the pursuer and the retinal target size. It is therefore particularly interesting to find out whether these visual parameters are represented explicitly at the neuronal level by the MLGs. Possibly these specific visual parameters are encoded separately by different male specific neurons. In my study, I wanted to test this hypothesis by investigating the response properties of one prominent male-specific visual interneuron, the MLG1

neuron, under visual stimulus conditions that come as close as possible to the visual input experienced by male flies during real chasing flights.

To determine the coding quality of MLG1 for specific visual cues characteristic of chasing situations, I confronted this neuron with naturalistic visual stimuli, as they are experienced by male flies during chasing of a target. Since nervous systems have evolved under natural conditions to compute behaviourally relevant information, they need to be studied not only with relatively simple stimuli conventionally used for systems analysis, but also from the perspective of freely moving animals (Zeil et al., 2008). Recent studies indicate that taking the natural stimulus conditions into account can be essential for an understanding of the functional relevance of neuronal processing and computations (e.g. Boeddeker et al., 2005; Burton and Laughlin, 2003; Kayser et al., 2004; Kern et al., 2005; Reinagel, 2001; Simoncelli, 2003; Simoncelli and Olshausen, 2001; van Hateren, 1997; van Hateren et al., 2005). Up to now it is not possible to carry out electrophysiological recordings from a neuron in a fly's brain while the animal is freely flying around. Therefore, I combined behavioural and electrophysiological experiments to obtain naturalistic stimuli as they are experienced by chasing males. To obtain these naturalistic stimuli, I first conducted behavioural experiments with freely flying male *Lucilia* that chased a dummy target (Fig. 2.2). The flight trajectories of the chasing males were recorded with high-speed digital video cameras. On the basis of these data the retinal images of the pursued target during a chase were reconstructed frame by frame (Boeddeker et al., 2003). These image sequences were then replayed on a monitor screen to the male fly that was tethered in the equipment for electrophysiological recordings. Thus, it was possible to carry out *in vivo* intracellular electrophysiological recordings from the MLG1 neuron while the male watched a movie that simulated the animal chasing a target.

The characterisation of the male-specific MLG1 neuron with naturalistic motion sequences reveals that this visual interneuron has a large receptive field that is located in the dorsofrontal region of the retina (Fig. 2.11). The receptive field of MLG1 thus covers most of the retinal area where the target is fixated during pursuit. MLG1 responds well to stimuli that extend into the contralateral visual field. These findings confirm earlier conclusions obtained with relatively simple stimuli that MLG1 does not only receive input from the ipsilateral eye, but also from at least one contralateral neuron (Fig. 2.11) (Beersma et al. 1977; Wachenfeld, 1994). MLG1 responds with graded shifts in membrane potential, often superimposed by spike-like depolarisations. The neuron exhibits a distinct direction selectivity and it responds best to visual motion stimuli that contain upward components and that move in the dorso-frontal area of the visual field, which is in accordance with previous studies (Gilbert and Strausfeld 1991; Wachenfeld 1994). Large neuronal depolarisations are evoked by motion in the preferred direction mainly within the centre of the receptive field. Moreover, depolarisations occur for both horizontal motion directions, i.e. for clockwise and counterclockwise motion. MLG1 shows no

pronounced inhibition during null-direction motion.

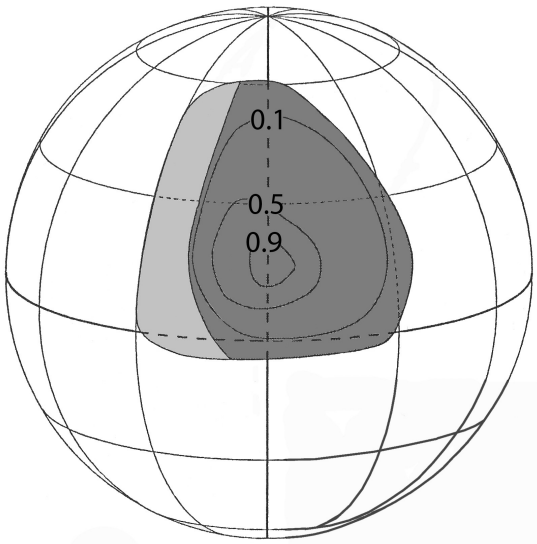

Figure 2.11 The receptive field of MLG1 (Wachenfeld, 1994). The receptive field of the MLG1 neuron is plotted on the polar coordinate system of the fly's visual field (for explanation of the polar coordinate system of the fly's visual field, see Fig. 4.1). The receptive field spans a large region of the ipsilateral fronto-dorsal visual field (*dark grey*) and extends far into the contralateral visual field (*light grey*). The sensitivity of the MLG1 neuron was measured by moving a small dot (5°x5°) on different traces across the visual field. The cell's sensitivity is given by isopotential lines that connect neuronal responses of the same strength to dot motion if normalised to the maximum neuronal response. The *numbers* represent the ratio of the response strength to the maximum response. Large parts including the most sensitive part of the receptive field of MLG1 spans the region of the male acute zone (compare Fig. 2.8).

The naturalistic visual stimuli are characterised by simultaneous variation of several visual parameters over time (Fig. 2.12). I tested the hypothesis whether retinal target position and/or retinal target size are represented explicitly by the MLG1 responses. A coherence analysis between the neuronal responses and any of these retinal stimulus parameters indicates that each of these individual cues is somehow represented in the responses of MLG1; however, there is no strong linear dependence of the neuronal responses on one of these stimulus parameters. These findings suggest that the responses of MLG1 do not explicitly represent either the retinal size, the position of the target or its velocity. Rather, MLG1 shows complex nonlinear response characteristics to the joint occurrence of multiple visual parameters indicating that size, position and velocity and their variation over time jointly affect the responses of MLG1. The combination of the preferred stimulus conditions of the MLG1 neuron are: target motion direction containing an upward component, high motion velocities of 500-2000°/s, duration of target motion for more than 20 ms and the position of target motion within the most sensitive region of the receptive field. These motion sensitivities and receptive field properties of MLG1 are in accordance with results as obtained with simple stimuli (i.e. moving

bars and small targets) that were as well employed in my study. Furthermore, these results corrobate a previous study that conducted very detailed characterisations of two MLGs, the MLG1 and the MLG2 neuron (Wachenfeld, 1994).

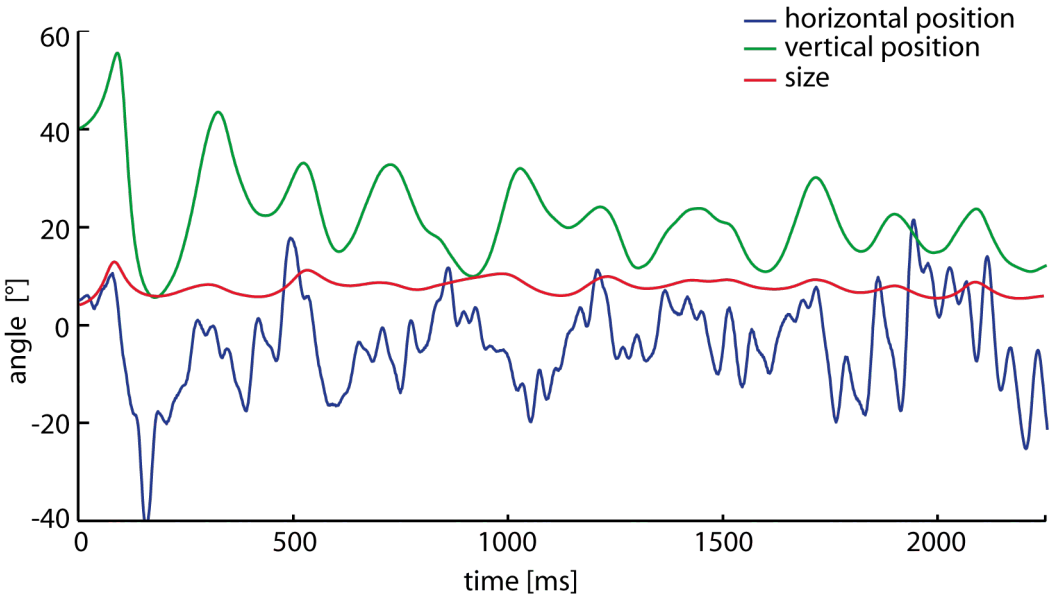

Figure 2.12 Time-dependent variation of the target's size and position on the retina of a chasing fly. These data were reconstructed from a video sequence of a male blowfly chasing a target that moved on a circular track. The *red line* denotes the retinal size, the *blue and green line* denote the centre of the target's horizontal and vertical position on the retina, respectively, as experienced by the male fly during the chase. While chasing, the target is well fixated within the eye region that roughly corresponds the area of the acute zone (compare Fig. 2.8) and the region that is subtended by the visual field of the MLG1 neuron (compare Fig. 2.11).

The preference for high motion velocities as found in MLG1 are also described at a very early level of the visual processing pathway in male flies: Photoreceptors in *Musca* were confronted with visual stimuli resembling changes in light intensity experienced by photoreceptors if targets, such as a conspecific, passes by. Electrophysiological recordings from the photoreceptors show that the gain of male acute zone photoreceptors is 3-4 times the female value. When presenting targets with different angular velocities, the optimum velocity for males is around 1000°/s, while this value is around 10-30°/s for females (Burton and Laughlin, 2003). Also the basic cell signalling processes within male (*Musca*) acute-zone photoreceptors are faster than that in females (Hornstein et al., 2000). These male-specific qualities are likely to enhance the ability to locate and pursue small fast-moving targets such as conspecifics.

What may be the functional relevance of the MLG1 neuron within a chasing controller? From the present knowledge it is probable that MLG1 plays a role in processing visual information within the chasing pathway (Gilbert and Strausfeld

1991; Wachenfeld, 1994) without exclusively signalling target size or position. Presumably the visual system of males solves the problem of processing the relevant information as occurs during chasing flights by other means than by signalling distinct target parameters separately.

When an animal moves, the experienced visual input that is shaped by behaviour should be 'matched' by the design of the underlying neuronal substrate that processes the visual images (e.g. O'Carrol et al., 1997). Eventually, the response properties of MLGs reflect the dynamic visual input as received by males during the high-speed virtuosic chasing behaviour. So far, I have not taken into account the other male specific neurons (MLGs and MCols). It is plausible to assume that a chasing controller may employ the whole ensemble of these neurons encoding the visual input during chasing behaviour. The division of the blowfly chasing control system into distinct pathways, exclusively signalling separate visual target parameters may be convenient for analytical reasons, but seems to get blurred at the neuronal level.

2.2.3 SMALL-FIELD SENSITIVE NEURONS IN TWO OTHER FLYING INSECT SPECIES

The ability of visual detection and pursuit of small moving targets is a common task of animals that search for prey or for a conspecific. The neuronal substrate subserving small target detection and pursuit was subject to investigations especially in hoverflies and dragonflies. However, while the chasing of conspecifics is exclusively a sex-specific behavioural task of male hoverflies, the pursuit of prey in dragonflies is evidently a sex-independent behaviour.

Male and female dragonflies are visual predators that pursue other flying insects for food. Especially dragonflies of the family Aeschnidae are very virtuosic aerobatic foragers that are able to fly speeds near 10m/s and that hover and manoeuvre in virtually all directions (Frye and Olberg, 1995). Males and females have a dorsal acute zone that is thought to be used for prey capture. The fast-flying Aeschnids have the largest eyes of all insects and impressive acute zones (Land, 1997). One species of this family, *Anax*, has the smallest interommatidial angles of any insect: 0.24° in the dorsal acute zone. This zone provides a narrow band of high resolution that is easily visible as a wedge of enlarged facets. O'Carroll (1993) described in the dragonfly *Hemicordulia* several classes of visual neurons in the third visual neuropil, the lobula. Some of these cells respond selectively to small moving targets that subtend visual angles equivalent to one or two facets of the compound eye (1°-2°). These neurons exhibit distinct direction selectivities and show no responses to large-field motion. Furthermore, intracellular recordings from descending neurons in *Anax* reveal that these cells are strongly direction selective (Frye and Olberg, 1995). Moreover, some of these cells are small-field sensitive and have a relatively small

visual field within the dorsofrontal eye region.

Hoverflies are described to be sophisticated flying artists and they exhibit a variety of flight behaviours. The males engage in precisely controlled virtuosic chases of conspecifics (Collett and Land, 1975). It is therefore not surprising that the eyes of males have an acute zone with enlarged facets, high resolution and small interommatidial angle of 0.6°. Recently, neurons in the male hoverfly *Eristalis* were found to respond with extreme selectively to small moving targets (Barnett et al., 2007; Nordström et al., 2006). These small target motion detectors (STMDs) exhibit clear direction selectivities and the receptive fields of these cells subtend the dorsofrontal eye region. Stainings reveal similarities to identified male-specific MLGs and MCols in *Calliphora*. The task of chasing may be especially demanding when the target is pursued in front of a structured environment, such as bushes or trees. Then, the moving pursuer needs to analyse targets against the motion of background clutter. Some of the STMDs in male hoverflies are found to respond even when target and background move at the same speed. This small-field tuning suggests that STMDs are inhibited by large-field (i.e. background) motion (Barnett et al., 2007; Nordström et al., 2006). It is fascinating that some of the STMDs even respond to very small targets (0.2° square), which is smaller than the size of the visual field of single photoreceptors (Nordström et al., 2006). Recently, movies of natural scenes containing a visual target were presented to acute-zone photoreceptors in male *Eristalis* (Brinkworth et al., 2008). Intracellular recordings from these photoreceptors reveal that target detection against a background begins already at this early level of visual processing (Brinkworth et al., 2008).

Apart from the latter study employing naturalistic stimuli analysing photoreceptors, the investigations of the small-field sensitive neurons in dragonflies and hoverflies have so far been carried out with relatively simple visual stimuli. These artificial 'laboratory' stimuli are conventionally used and can be essential for systems analysis. However, these stimuli differ considerably from naturalistic visual cues as they are experienced by the animals in natural behavioural situations. Using naturalistic stimuli, the functional relevance of the neural substrate can also be studied from the perspective of freely moving animals. In my study, the use of naturalistic chasing stimuli indicated that the male-specific MLG1 neuron responds best to the joint occurrence of multiple visual parameters of the target rather than to just one particular parameter. Employing natural stimulus conditions for examinations of small-field motion sensitive cells in other insects may as well contribute to a further understanding of the functional relevance of the processing and computations of these cells.

2.2.4 NEURONAL SUBSTRATE UNDERLYING THE OPTOMOTOR SYSTEM OF FLIES

As animals move through the world, they experience a distinct pattern of continuously changing images on the retina. For course stabilisation, many animals use the so-called optic flow (e.g. Zanker and Zeil, 2001). Optic flow processing in the fly is carried out by about 40-60 so-called tangential cells that are localised in the lobula plate. Tangential cells have large receptive fields and are assumed to be tuned to different types of optic flow (reviews: Borst and Haag, 2002, Egelhaaf et al., 2002; Hausen and Egelhaaf, 1989; Krapp, 2000).

Three of these tangential cells of the horizontal system (HS) in each hemisphere of the fly brain are supposed to be the neuronal substrate underlying the fly's optomotor response (Fig. 2.13; review: Hausen and Egelhaaf, 1989). The main response mode of these cells to large-field horizontal (ipsilateral) image motion from front to back is a graded depolarization. Due to their response characteristics, HS-cells were originally thought to act primarily as rotation detectors (Egelhaaf and Borst, 1989; Hausen, 1982a; Hausen, 1982b; Hausen and Egelhaaf, 1989; Warzecha and Egelhaaf, 1996). These HS-neurons are assumed to provide input to the rotation-sensitive optomotor system.

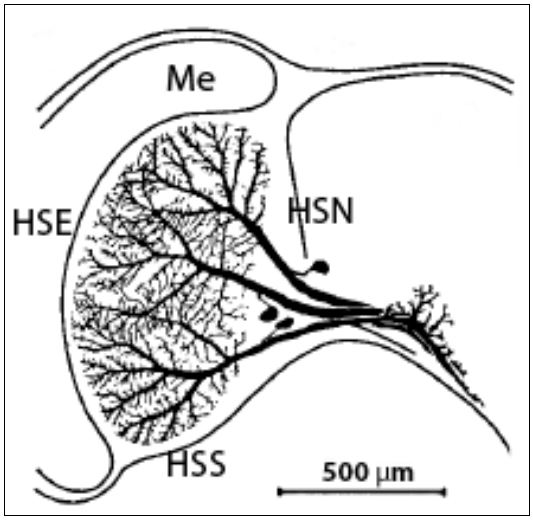

Figure 2.13 Dendritic branching patterns of the HS neurons (Hengstenberg et al., 1982). Three wide-field tangential neurons in the lobula plate constitute the horizontal system (HS). According to their positions the three HS cells are called *HSN* (north), *HSE* (equatorial) and *HSS* (south). The dendrites of the three HS cells each extend over roughly 1/3 of the lobula plate. *Me* Medulla.

In many previous studies, the optomotor response was elicited in tethered flies by rotating a structured panorama around the animals. Recent electrophysiological studies on the fly's HS-system employed naturalistic optic flow, as experienced in free flight manoeuvres. In contrast to classical optomotor stimuli that were commonly used to investigate HS-cells, the naturalistic optic flow is shaped by the saccadic flight and gaze strategy that is characteristic of cruising flight (see chapter 2.1.4). As a consequence, naturalistic optic flow is characterised by sequences of brief rotational segments, resulting from the saccades, and longer translational

segments that result from the straight flight and constant gaze direction during the intersaccadic intervals (cf. Fig. 2.6). The studies using naturalistic optic flow as visual stimulus show that the HS-responses can provide information during the intersaccadic intervals about translational optic flow information and thus about the three-dimensional structure of the environment (Boeddeker et al., 2005; Kern et al., 2005, Lindemann et al., 2005; van Hateren et al., 2005, Karmeier et al. 2006). Hence, the functional role of HS-cells is likely to be more complex than anticipated by the classical studies that considered only optomotor following responses.

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3 Chasing behaviour and optomotor response in free-flying male blowflies: Flight performance and interaction of the underlying control systems.

3.1 INTRODUCTION

Much of our motor activity is guided by what we see. Examples are visually guided grasping or tracking of an object. Several components of visuo-motor behaviour of invertebrates and the eye movements of vertebrates including humans have been examined in considerable detail throughout the last decades (e.g. Port et al., 1997; Smeets and Brenner, 1995; van Donkelaar et al., 1992; reviews: e.g. Carpenter, 1988; Land, 1992; Land, 1999). The generation of visually guided movements involves processing of sensory stimuli and transforming them into central commands that may eventually control the activation of the relevant musculature.

In humans, visual pursuit of a small target by eye movements may be employed for solving various tasks such as catching baseballs or watching a race (e.g. Jacobs et al., 1996; Land and McLeod, 2000; Land and Tatler, 2001; McBeath et al., 1995; Shaffer and McBeath, 2002). In animals visual pursuit may not only involve eye movements, but also movements of the head or the entire body towards the pursued target. For instance, predators may use visual cues connected with their prey to pursue and catch it, for instance jumping spiders (Jackson and Pollard, 1996), dragonflies (O'Carroll, 1993; Olberg et al., 2007), praying mantids (Rossel, 1980), archer fish (Rossel et al., 2002) or dogs (Shaffer et al., 2004). Visual pursuit may also be performed in the context of mating behaviour, i.e. when males try to catch females in order to mate. Examples are, for instance, hoverflies (Collett and Land, 1978), blowflies (Boeddeker et al., 2003), houseflies (Land and Collett, 1974; Wagner, 1986b; Wagner, 1986c) and drone bees (e.g. Vallet and Coles, 1993; van Praagh et al., 1980).

The fixation of a stationary object during self-motion is related to visual tracking of a moving object, since the object would move across the retina if it were not fixated by eye, head or body movements. Fixation behaviour is not only relevant for humans (e.g. Land, 1999) but also, for instance, for flying animals, such as blowflies (Kimmerle et al., 1997) and houseflies (Egelhaaf, 1987) that fly towards and land on an object such as a flower.

These examples are all concerned with the fixation and visual pursuit of relatively small objects (i.e. small-field stimuli). In vertebrates (including humans) and invertebrates visual following responses emerge as well in response to motion of the entire visual field (i.e. large-field stimuli), such as during self-motion in a structured

environment. For instance, while sitting in a moving train and looking out of the window the eyes inevitably show optokinetic responses (OKN, optokinetic nystagmus) that follow the moving landscape (reviews: e.g. Ilg, 1997; Miles, 1993). By preventing the slip of the visual image by eye movements the control system underlying optokinetic responses helps to stabilize the position and orientation of the eyes in space, such as during self-motion (Miles and Wallman, 1993). Similar optokinetic (i.e. optomotor) following responses to large-field motion of the head and/or body are found in invertebrates, for instance in flies (e.g. Götz, 1975; Warzecha and Egelhaaf, 1996) and are as well implemented in bio-mimetic artificial systems (e.g. Webb et al., 2004).

How the visual information is processed during following responses by the nervous system has been studied experimentally and by model analysis especially in primates and in flies (Keller and Missal, 2003; reviews: e.g. Carandini et al., 2005; Taylor and Krapp, 2008; Zeil et al., 2008). In the present study, blowflies (*Lucilia sp.*) were used as experimental animals to analyse both pursuit of moving objects as well as optomotor following response and how these two components of visuo-motor behaviour may interfere with each other. To understand why this question is interesting, let us assume the situation that an animal, such as a male blowfly, pursues a conspecific and thereby makes intentional turns toward its target. Target-induced turns into a given direction in front of a structured background inevitably lead to large-field motion in the opposite direction. This large-field motion may then evoke a following response into the opposite direction of the intended turn and, as a consequence, impede the pursuit of the moving target. Thus, during chasing in a natural environment the two visually driven following behaviours may be in conflict with each other. In particular, it may be important for flying insects to be able to distinguish self-induced visual motion, such as large-field retinal image displacements caused by following a target, from unintentionally externally imposed visual motion, such as retinal large-field motion due to a gust of wind.

The interaction of two potentially conflicting control systems is not restricted to flies. Rather, it is a quite general problem that is likely to concern all moving animals including humans as well as autonomous artificial agents such as robots with visually guided large-field and small-field response behaviours. Mechanisms have been proposed in previous studies by which compensatory following responses do not continually counteract voluntary turns. One of the first models for such a mechanism is the 'efference copy' model (von Holst and Mittelstaedt, 1950) or 'corollary discharge' scheme (Sperry, 1950). Here, a copy of every motor command is internally generated corresponding to the expected visual input resulting from the motor action. This 'efference copy' is sent to the optomotor system in order to cancel out the neuronal responses to the expected visual consequences of the turning command. In addition to these 'classical' schemes Collett (Collett, 1980a) proposed two further models that model the interaction between a pursuit system and the optomotor following responses. In the 'follow-on' scheme, the turning command of

the chasing system is injected into the optomotor loop and thus the optomotor system executes the desired turn towards the target. In the 'additive model' both control systems are independent until their commands come together at a final pathway. Both these schemes have some biological support (e.g. Virsik and Reichardt, 1976; von Holst and Mittelstaedt, 1950; Wagner, 1986c). It seems plausible that different animals might solve the problem of distinguishing between the visual consequences of intended movements and unintended external ones in somewhat different ways, dependent on the behavioural context (review: Crapse and Sommer, 2008).

It is the one major goal of my study to investigate the impact of the optomotor system on the performance of chasing behaviour. Before addressing this topic in more detail, a short overview will be given in the following about the two concerned behavioural components, the blowfly's chasing behaviour and its optomotor behaviour, as well as the corresponding underlying control systems.

Pursuit of a small target

Chasing behaviour of male flies is one of the fastest visually guided behaviours that can be found in nature. Males but not females of several species of flies pursue other individuals in the context of mating behaviour with high virtuosity and high accuracy (Collett, 1980a; Collett and Land, 1975; Collett and Land, 1978; Land 1993a; Land, 1993b; Land and Collett, 1974; Wagner, 1986a; Wagner, 1986b; Wagner, 1986c; Wehrhahn, 1979; Wehrhahn et al., 1982; Zeil, 1983). Male flies have anatomical and physiological specialisations within the forward directed eye region and at several levels of the corresponding parts of the visual system. These specialisations are likely to enhance the performance in chasing behaviour (Gilbert and Strausfeld, 1991; Gronenberg and Strausfeld, 1991; Hornstein et al., 2000; Land, 1997; Land and Eckert, 1985; Nordström et al., 2006; Strausfeld, 1991; Trischler et al., 2007). Recently, a model of the *chasing control system* in male blowflies has been shown to use the retinal size, the retinal position and the velocity of the target as input variables (Boeddeker and Egelhaaf, 2003; Boeddeker and Egelhaaf, 2005; Boeddeker et al., 2003). In insects, pursuit of a small object can be accomplished by smooth movements of the body or of the head since the eyes are fixed to the head capsule.

In primates smooth pursuit is characterised by continuous rotations of the eyes, sometimes in combination with head and body movements (Krauzlis, 2004; Miles, 1997; Schweigart et al., 1997). Vertebrates and invertebrates fixate the visual target during smooth pursuit thus minimizing the blur that would otherwise comprise visual acuity (Land, 1999; Westheimer and McKee, 1975). Nevertheless, pursuit responses are not exclusively smooth; pursuit can be quite jerky when intermitted by rapid gaze shifts, the so-called saccades. Saccades are discrete movements that quickly change the orientation of gaze, thereby translating the image of the object of interest from an eccentric retinal location to the focus of gaze. Saccades are rapid eye

movements in primates, and are fast orientation changes of the head and/or the body in insects.

The experimental animals used here, the blowflies, have been shown to employ both smooth and saccadic pursuit: Whereas smooth chasing is thought to keep the image of the target fixated in the frontal visual field, rapid saccadic body turns serve to recenter the moving object whenever it deviates too much from the retinal fixation region (Boeddeker et al., 2003; Collett, 1980a; Land 1993a; Land and Collett, 1974; Wagner, 1986b; Wehrhahn ,1979; Zeil, 1983; review: Land, 1992). Recently, a phenomenological model of the chasing system in blowflies revealed that both smooth pursuit and apparently saccadic pursuit can be accounted for by a single control system (Boeddeker and Egelhaaf, 2003). If the target is displaced rapidly on the pursuing fly's retina, the so-called catch-up saccades help to centre the target on the frontal eye region.

Similarly, in primates, including humans, a smoothly moving small target normally evokes, depending on target velocity, a combination of smooth and saccadic eye movements. Behavioural and neurophysiological data demonstrated that both types of eye movements work in synergy to accomplish visual tracking (review: Orban de Xivry and Lefèvre, 2007). At low target speeds (<50°/s), the target is kept fixated in the fovea by slow and smooth eye movements. When the target is displaced outside the fovea, or when the target motion is too rapid, smooth pursuit is interrupted by saccades to centre the target again (Rashbass, 1961; reviews: Carpenter, 1988; Land, 1992; Land, 1999). This ability distinguishes primates from other vertebrate species such as rabbits and fish, which make smooth eye movements only when the entire visual scene is moved as during the OKN. None of these animal groups has a strong pursuit system for small-field stimuli (Lisberger at al, 1987). Only recently smooth pursuit of small targets has also been revealed in cats (de Brouwer et al., 2002a; de Brouwer et al., 2002b).

Optomotor following in flies

Optomotor following responses are assumed to compensate under normal free-flight conditions for external disturbances as well as for internal asymmetries of the animal (Collett et al., 1993; Hengstenberg, 1993; Kern and Egelhaaf, 2000; Strauss and Heisenberg, 1990; Wehner, 1981). They have been examined in great detail in tethered flight (reviews: Egelhaaf and Borst, 1993; Hengstenberg, 1993; Heisenberg and Wolf, 1984; Reichert, 1993) as well as in free flight not only in flies (Collett, 1980a; Collett, 1980b; Duistermars et al., 2007; Frye and Dickinson, 2007; Land and Collett, 1974; Tammero and Dickinson, 2002a; Wagner, 1986a; Wagner, 1986b; Wagner, 1986c). When flies are confronted with a large-field rotating stimulus, they may try to compensate for this rotation (simulating an unintended self-rotation) by turning responses in the direction of the moving visual stimulus. The visual system of flies integrates the global retinal image displacements, the so-called optic flow, evoked during their flight manoeuvres. The optic flow is evaluated by the visual

system and plays an important role in controlling the flight path (reviews: Collett et al., 1993; Egelhaaf and Borst, 1993; Egelhaaf and Warzecha, 1999; Reichert, 1993; Egelhaaf, 2005). The response properties of visual interneurons in the blowfly brain processing optic flow information and, in particular wide-field motion as induced during rotations of the animal were studied extensively in flies (e.g. Hausen and Egelhaaf, 1989; Krapp and Hengstenberg, 1996; Krapp et al., 2001; Egelhaaf, 2005).

Interaction between optomotor following responses and target pursuit

The possible impact of optomotor stimuli on the performance of target pursuit was investigated in several studies in diverse insect species. For instance, in mantids the pursuit response to a small target is strongly affected in front of a structured stationary background (Rossel, 1980). In the hoverfly *Syritta* the chasing behaviour has been shown to be influenced by a simultaneously presented large-field optomotor stimulus (Collett, 1980a). By contrast, in the housefly *Musca*, optomotor stimulation had no obvious influence on the chasing behaviour (Wagner, 1986c).

Also in primates the issue of possible interactions between visual pursuit and large-field following responses has been intensively studied, but is still controversial: On the one hand, visual pursuit is used by the eyes to track accurately small objects of interest. On the other hand, during self-motion, visual large-field stimuli evoke the optokinetic nystagmus (OKN), a reflex that corresponds to the fly's optomotor response and prevents the slip of the visual image by evaluation of large-field image movement (reviews: e.g. Keller and Heinen, 1991; Land, 1992; Lisberger et al., 1987). Many studies done on primates (including humans) examined the influence of a stationary or a moving textured background on pursuit of a moving target and revealed various significant effects: On the one hand, the steady-state pursuit eye velocity (induced by a small moving target) was found to increase when the background moved in the same direction as the pursued target and was decreased when the background moved in the opposite direction as the pursued target (Masson et al., 1995). On the other hand, Schwarz and Ilg found that brief background shifts opposite to the target motion direction do not alter the performance of target pursuit. In contrast, those in the same motion direction resulted in a transient perturbation of the pursuit (Schwarz and Ilg, 1999). Other behavioural studies indicate that pursuit eye movements are variously affected by a stationary or dynamic visual background (Collewijn and Tamminga, 1984; Keller and Khan, 1986; Kimming et al., 1992; Masson et al., 1995; Mohrmann and Thier, 1995; Niemann and Hoffman, 1997). Despite the inconsistencies between these studies, they indicate that in primates the control systems mediating large-field following and small-field pursuit do not work independently of each other, at least at the behavioural level.

Outline

Based on behavioural experiments done with almost freely flying blowflies, my study aims (1) to characterise the behaviours and the underlying control systems

that guide visual target pursuit and the optomotor following. This is done by employing high-resolution digital video techniques and analysing the detailed time structure of the flight trajectories. As a reference for the two visually driven behavioural components cruising flights were employed, i.e. spontaneous flights that do not serve any obvious purpose. In addition to characterising both systems in isolation, (2) the potential interaction between optomotor and the chasing system will be analysed in the same experimental setup. The behavioural experiments were designed to stimulate the two control systems simultaneously: The visual environment of chasing male flies was manipulated (i.e. moved), thus presenting optomotor stimulation that was increased or decreased with reference to the normal stationary environments. Furthermore, since the flight behaviour of flies have been examined in previous studies mainly in females (see above), in my study the results in male and female blowflies will be compared. Finally, I will discuss my results in the context of concepts proposed previously to explain the interaction between chasing and optomotor following.

3.2 MATERIALS AND METHODS

Experimental setup

All experiments were conducted with several sets of blowflies (*Lucilia sp.*), whereof one set consisting of about 20 flies was used over several days to conduct all different types of behavioural experiments with the same animals. All experiments were done with males, with the exception of the last experiment ('Are there sex-specific differences during cruising flights?') which was carried out with one set of males and a separate set of females. The flies (6-10 day-old) were released in a cylindrical flight arena (radius 0.2m, height 0.7m), the round side-wall and the floor of which consisted of clear Perspex; the ceiling was homogeneously white. The arena was illuminated, on the one hand, by the illumination provided by the green LEDs of the stimulus device. On the other hand, to deliver enough light for the video cameras, two Tungsten light heads (DLH4, 150 Watts, Dedo Weigert Film, Germany) additionally illuminated the arena from the bottom. To ensure that these lamps do not much reduce the contrast of the visual stimulus and because red light should be relatively invisible for blowflies (Francescini et al., 1981; Hardie, 1979), both lamps were endowed with a dichroic red-light filter (DFCOL2R, Dedo Weigert Film, Germany) in their front. For the duration of the experiments, the temperature in the flight arena ranged between 20°C and 29°C. All experiments were done in a darkened room.

Visual stimulation

The arena was surrounded by a panoramic stimulus device that consisted of 20 printed circuit boards, two lines of ten boards each stacked over each other. Each

board (0.15m high) contained 48 columns and 30 rows of single LEDs (2.5 x 5 mm^2). The sampling rate was 200 Hz. Each column could be switched on and off independently. The horizontal angular extent of each LED column amounted to 0.75°. The time until an LED reached a constant luminance value after switching on or off was 20-50µs. The device was programmed to generate an apparent motion of a vertical grating pattern moving horizontally. The periodic square-wave gratings had a spatial wavelength of 15°. Generating one frame, i.e. addressing all groups of LED-columns serially, took approximately 370µs. The cylindrical LED-array spanned 330° in azimuth and 60° in zenith as seen by the centre of the arena. Flies flying in the arena appeared rather dark in the black-and-white camera images (Fig. 3.1). To enhance the contrast of the fly against the dark bars of the grating in the field of view of the lateral camera (see below), a correspondingly sized parchment paper was placed in front of the respecting grating patch. The contrast amounted to 25% in front of the parchment paper and 85% in the residual arena (Michelsen contrast).

Within the different types of experiments two types of visual stimuli were used: a dummy fly and a visual background. A black sphere (diameter: 5mm) served as dummy fly (Fig. 3.1). It was attached to the tip of a Perspex stick and was moved clockwise on a circular track (radius: 80mm) in a horizontal plane about 100mm beneath the ceiling of the arena. The speed of this dummy target was 1m/s (i.e. about 700°/s), which resides within the speed range of real flies.

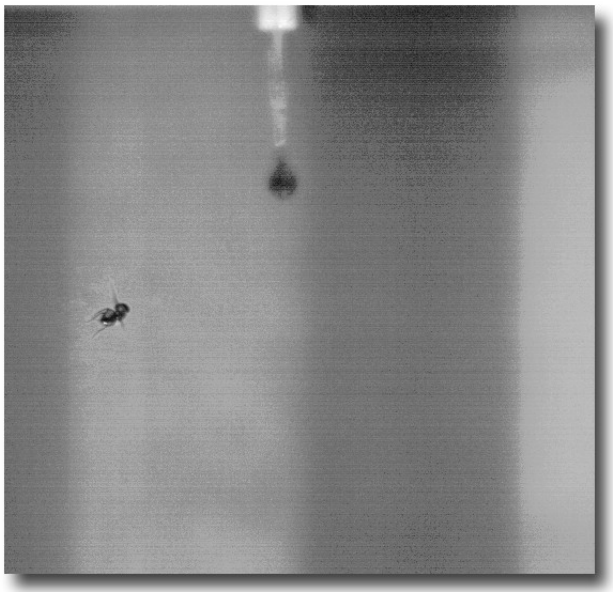


Figure 3.1 Photo of a male blowfly chasing the target. A fly is chasing the 'female dummy fly' which is a black sphere (diameter: 5 mm). This target resided on the tip of a Perspex stick (length: 65mm). The Perspex stick was attached to the ceiling which could be rotated in the horizontal plane. In the back can be seen the vertical grating pattern that was generated by illumination of LEDs. Note that the blur of the grating (background) results from inhomogeneous illumination of the parchment paper by the red-light Tungsten lamps.

The grating pattern was either stationary or rotated horizontally at four different velocities. Hence, five different background conditions were tested. First condition: As a reference, the grating was held stationary (0°/s). Second to fifth condition: The grating was moved clockwise at two velocities (45°/s or 365°/s) as well as counterclockwise at the same two velocities. The slow pattern motion corresponds

to a temporal frequency of 1.5Hz, whereas the fast motion corresponds to a temporal frequency of 12Hz. The flight behaviour of the flies in different contexts was filmed and stored for the further analysis. (1) The flies performed cruising flights in front of a stationary grating pattern as background. (2) To record optomotor flights, the background pattern was moved clockwise with two different velocities, either slowly (45°/s) or quickly (365°/s). While recording cruising and optomotor flights, the dummy target was removed from the flight arena. (3) While recording chases, the target was moved clockwise on a circular track. Simultaneously, the visual environment was manipulated by presenting the grating (Fig. 3.1) as characterised for the five different background conditions (see above). Chases recorded in these experimental paradigms were used on the one hand for the analysis of the flight trajectories, on the other hand for analysing the 'catching frequency' (i.e. the number of catches occurring within a distinct time window), and finally for analysing the 'flight frequency' (i.e. the number of flights that were performed within a distinct time window).

The procedure of the catching-frequency experiments was as follows: The illumination (i.e. illumination by the lamps and the LED-device) was changed between light and darkness (Fig. 3.2A; 70s light on, approximately 150s light off). About 15s after the illumination has been turned on following a dark period, the flies gradually began to fly again, and the number of flying flies appeared to reach a kind of steady state within the next 15s. Thus, 30s after the illumination has been turned on, the catching frequency was evaluated for the following 40s.

The procedure for the flight frequency experiments consisted of similar changes between illumination and darkness (Fig. 3.2B; 60s light on, about 150s light off). About 30s after the illumination has been turned on, the number of flying flies appeared to reach a kind of steady state. The flight frequency was evaluated in the following 30s time-window.

Video analysis

Within the scope of my study, two different types of video analysis were employed. On the one hand, for the analysis of the flight frequency I was not interested in characterising the entire flight sequences. This analysis therefore could be done with a conventional video camera (CCD Video Camera; VCB-3512P; Sanyo, Japan; frame rate 50 Hz, PLL 2:1, 795x596 pixels) that was positioned underneath the arena and viewed the entire aperture of the arena. On the other hand, since I also wanted to characterise the flight parameters in detail, flies were filmed with two orthogonally arranged digital high speed cameras (MotionPro 500, Redlake, San Diego, CA., spatial resolution: 1024 x 1024 pixels^2). One camera was positioned besides the arena viewing the upper part of the arena from the side through a gap in the stimulus device. The other camera was placed underneath the arena and covered the entire aperture of the arena. For the analysis of the time structure of the flight trajectories, the chases were filmed at a sampling rate of 250Hz.

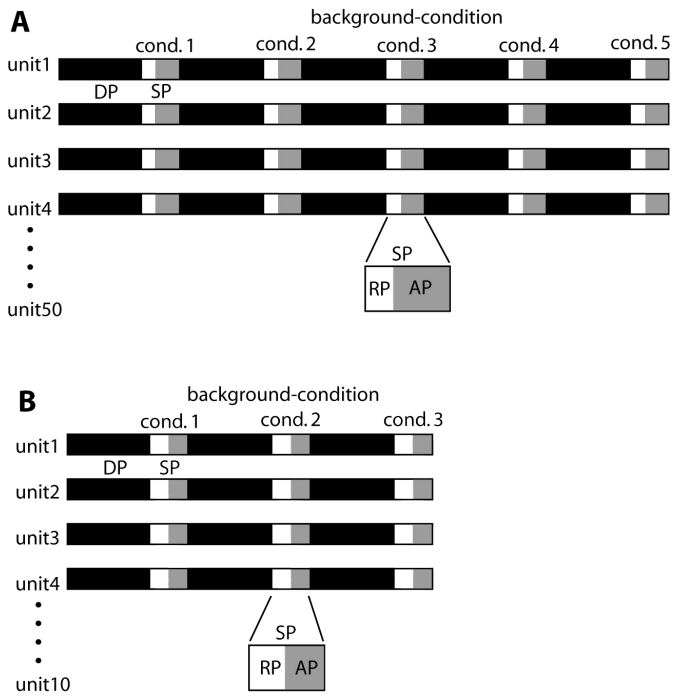


Figure 3.2 Time schedules of the data acquisition procedure. **(A)** Determining the catching frequency of the target by male flies. **(B)** Determining the flight frequency of male flies. **(A)** The target dummy moved during the entire experiment on a circular track at 700°/s. Within the whole stimulation period (*SP*, duration 70s; see *Inset*), the illumination was switched on. The illumination of the flight arena is described in Methods. The background stimulus was generated by a large-field vertical grating that adopted one of 5 different conditions. It either was stationary (*cond.1*), or it moved at 45°/s clockwise (*cond. 2*), at 45°/s counter-clockwise (*cond. 3*), at 365°/s clockwise (*cond. 4*) or at 365°/s counter-clockwise (*cond. 5*). Each stimulation period was preceded by a dark period (*DP*, about 150 s) during which the flight arena was not illuminated. Within the dark period, the flies stopped flying and sat on the wall and floor of the flight arena. After the onset of the stimulus the flies gradually started flying again, and the number of flying flies appeared to reach a kind of steady state after about 30s. ***Inset***: After this 30s 'recovery period' (*RP*) followed the analysis period (*AP*), lasting 40s, within which I counted how often the target was caught. The experimental procedure was as follows: The five different background conditions were presented in the order as depicted in the first row (*unit1*). This procedure 'unit' was repeated 50 times (*unit 1-50*). **(B)** To determine the flight frequency, the illumination of the flight arena was switched on within the stimulation period (*SP*, duration 60s; see *Inset*). The illumination is described in Methods. In this experiment, only the numbers of flies that started to fly around in front of a stationary, slowly or quickly moving background were of interest. In this situation, only the velocity of the background motion was relevant, but not the relation of the target motion direction to the background motion direction. Therefore, within these experiments, three different background velocities accounted for the three different conditions: That are stationary background (*cond.1*), the background moving slowly at 45°/s (*cond. 2*), and the background moving quickly at 365°/s (*cond. 3*) in clockwise direction. Each stimulation period was preceded by a dark period (*DP*, about 150s) during which the flight arena was completely dark. ***Inset***: After the 'recovery period' (*RP*, 30s) followed the analysis period (*AP*) lasting 30s. I segmented the AP into 30 consecutive 1s time bins and I counted the number of flights occurring within each of these bins and calculated the averages over the number of flights across the 30 consecutive 1s bins within each analysis period. The experimental procedure was as follows: The three different background conditions were presented in the order as depicted in the first row (*unit1*). Such units were repeated 10 times (*unit 1-10*). During these experiments, about 30 male flies resided in the flight arena.

For the evaluation of the catching frequency, the chases were filmed with a sampling rate of 50Hz. The digital video sequences were stored as uncompressed 8-Bit AVI-files on the computer hard disk for off-line processing. For the analysis of the flight trajectories, the 2-D positions of the fly and the target, as well as the longitudinal body axis orientation of the fly were determined frame by frame with the aid of custom-built software, using standard image processing algorithms. Knowing the relative position of the two cameras, it is possible to transform 2D image coordinates into an orthographic 3-D coordinate system (Boeddeker et al., 2003; Zeil, 1983). I determined for each frame of the video the position and the orientation of the fly, and, in chases, the position of the target. The detectability of the fly and the target in video images is affected by inhomogeneous illumination of the flight arena, reflections on the fly's body and wings as well as lens aberrations of the camera objective. To assess methodological errors, the given position and orientation of a perched fly was reconstructed. The velocity error that is caused by the orientation determination was measured by reconstruction of the yaw velocity, and its standard deviation was 45°/s. The position error that is caused by distortions of the camera optics, increased with increasing eccentricity of the fly in the flight arena, but was always below 2 mm. The reconstruction of the 3D-trajectories and all further data processing and analysis was done in Matlab 6.5 (Mathworks, Natick, MA, USA).

Data analysis

Analysis of the catching frequency: Male flies chasing the target were filmed within 50 experimental units, whereof in each unit all five visual background conditions were presented subsequently as depicted in the first row for the first unit in Fig. 3.2A. After a dark period (about 150s), the illumination by the two lamps and the LED-device was switched on and the background stimulus was presented for the duration of the stimulus period (lasting 70s). Within this period, the flies had 30s to 'recover' flying in the illuminated arena. After these 30s of recovering followed the analysis period (lasting 40s), within which I counted how often the target was caught. If a fly succeeded to grab the target with its legs and sit thereon, this was regarded as a catch. Within each unit (of the 50 units), the overall number of catches across all background conditions was normalised. The question was if the catching frequency differs at various background conditions with reference to stationary background. The null hypothesis was that the medians of the catching frequency of two background conditions are equal. This hypothesis was tested using the Wilcoxon signed rank test of equality of medians.

Analysis of the flight frequency: Male flies flying in the arena and chasing the target were filmed within 10 units. For this analysis, I counted the number of flies that flew within the flight arena within certain time windows. Thereby, every fly that left its seat and started to fly, be it for chasing, or be it for flying around without any obvious goal, was of importance. In this situation, the relation of the target motion

direction to the background motion direction is not significant. Only the velocity of the background motion is relevant. Therefore, within these experiments, only three different background conditions were used: That are, first, stationary background, second, the background moving slowly (45°/s), and third, the background moving quickly (365°/s) in clockwise direction. Within each unit the three visual background conditions were presented subsequently (Fig. 3.2B). After a dark period (about 150s), the background stimulus was presented for the duration of the stimulus period (lasting 60s). This stimulus period consisted of a recovering time (lasting 30s), followed by the analysis period (lasting 30s). For analysis, the number of flies that were flying in the arena within each of the 30 of consecutive 1s time-bins were counted. I calculated the averages over the number of flights across the 30 consecutive 1s time-bins within each analysis period. I tested statistically if the number of flights differs in front of various background conditions (Kruskal Wallis Test).

For the analysis of the time-structure of the flight trajectories, the chases recorded at the five (as above) specified background conditions had to meet two criteria: First, the flights had to last for a minimum duration. Second, only chasing flights that are supposed to be 'real' chases should be included in the analysis. To meet the duration criterion, cruising as well as optomotor flights had to last for at least 150ms. For a part of the chases, this duration criterion differed: Chases were classified by their catching success according to successful chases in that the target was caught ('C-chases') after short time (mean duration=371ms, std=172ms, n=50) and unsuccessful chases in that the target was pursued ('P-chases') without catching it (Boeddeker et al., 2003). In P-chases the fly either approached but missed the target, or it gave up chasing and retired. Because of the obvious target capture, C-chases did meet the second criterion and therefore these flights could be included in the analysis when lasting at least 150ms. In P-chases, a differentiation was necessary between males that actually chase the target and males that coincidentally fly for some time in the same direction as the target. To be confident that the male actually chases the target, only those P-chases in that the male followed the target for at least one lap of the dummy (i.e. for at least 510 ms) were classified as 'real' chases and included in the analysis.

Within the recorded flight sequences I determined frame by frame on the one hand the 3-D position of the target, and on the other hand the 3-D position, the yaw orientation and, if possible, the pitch orientation of the fly. Additionally, I determined the fly's angular body orientation that provided an estimation of the fly's horizontal gaze direction in each frame. Due to methodological constraints, it was not possible in most situations to resolve the fly's head orientation which may be critical since blowflies can move their head relative to their body during flight (Hengstenberg, 1993; Land, 1973; van Hateren and Schilstra, 1999). However, rotations of the head about the pitch and roll axes are small during flight (Schilstra and van Hateren, 1998). (For further remarks, see chapter 3.4.) The pitch angle

could be determined only in the frames when the fly was entirely visible for the camera from the side. When viewing the fly either halfway or in forward or backward view, the pitch could not be resolved properly.

These data allowed the analysis of the flight trajectories with respect to the parameters of interest, which are the fly's yaw velocity, forward velocity, turning frequency, the distance to the target and the error angle. The error angle of the target is the fixation error in the azimuth relative to the frontal midline of the fly's visual field. It was calculated on the basis of the fly's body long axis by a line connecting the fly's longitudinal body axis and the target's centre, which represents an appropriate approximation of the error angle. To evaluate the frequency of the yaw turns that the males execute during flights, I needed to define what events of the yaw velocity profile can be classified as turns. Although the yaw velocity changes continuously, discrete events are defined as turns by applying several thresholds. First, the mean angular velocity is subtracted from the angular velocity trace. On these data, five thresholds were applied within the positive and negative domain (Fig. 3.3). The lowest threshold at 150°/s lies above three times the standard deviation of the methodological error (45°/s, see above). Very small velocity fluctuations that presumably represent noise lie below this threshold. During chasing, the fly may start another turn before 'terminating' the previous one (Fig. 3.3). In this case, the lowest threshold may not detect two separate turns, but only one (long-lasting) turn. Therefore, further thresholds at 300°/s, 500°/s and 800°/s are applied to the data. To detect turns characterised by large yaw velocities, a fifth threshold at 2000°/s was introduced. Moreover, the peak yaw velocity was calculated by measuring the maximum yaw velocity that was reached during each turn.

In chases, for the analysis of some parameters characterising flight or chasing performance (i.e. the mean yaw velocity, the peak yaw velocity, the mean forward velocity and the error angle), I did not evaluate the entire flight episode. The entire flight included behavioural patterns at the begin and at the end of a chase, and these are definitely essential parts of the chase. However, my study focuses on the analysis of the flies' chasing performance *during* the pursuit. Therefore, initial and final behavioural patterns were excluded from the analysis. For instance, many males start a chase with an initial turn towards the target (see Fig. 3.4A). This turn most likely serves to fixate the target within the frontal visual field. Hence, the first 60ms of each chase including the initial turn were omitted from the analysis. Similarly, at the end of a chase, before catching the target, the chasing fly changes its orientation in the horizontal plane. During this final turn the pursuer grabs the target with the legs often from the side (see Fig. 3.4A; and Boeddeker et al., 2003). The angle subtended by the fly's longitudinal body axis and the targets instantaneous flight direction was used as an indicator for the end of the chase. During the chase, this angle is around 0°. While the fly executes the final turn, this angle increases often up to 90° (Boeddeker et al., 2003). After this angle had reached the threshold of 20°, the residual flight episode (including the final turn) was excluded from analysis.

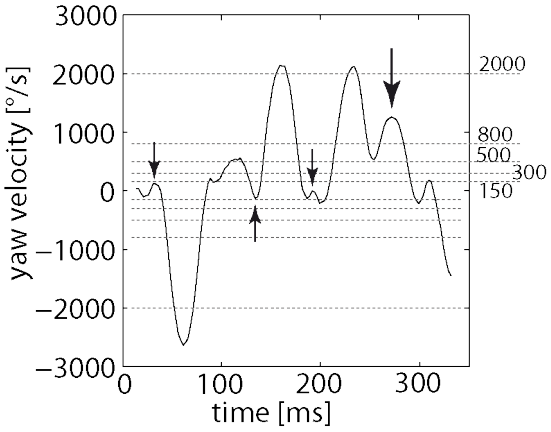


Figure 3.3 Procedure for the determination of turns. The yaw velocity profile was thresholded. This was done by subtracting the mean angular velocity from the entire yaw velocity profile, which is therefore centred around 0°/s. I applied to the positive domain of these yaw velocity data five thresholds at *150°/s, 300°/s, 500°/s, 800°/s* and *2000°/s* (*dotted lines*) and the corresponding negative values to the negative domain (*dotted lines*). The lowest threshold (at *150°/s*) lies above three times the standard deviation of the methodological error (see Methods). Very small velocity fluctuations (*small arrows*) that presumably represent noise lie below the lowest threshold and thus were not detected as turns. During flight, the fly may start a new turn before 'terminating' the previous one. This new turn 'rides' on the slope of the previous turn (*large arrow*). As can be seen, in this case, the lowest threshold may not detect two separate turns, but only one long-lasting turn. Therefore, the further thresholds at 300°/s, 500°/s and 800°/s were applied to the data which allow detecting both turns as two discrete events. To detect turns characterised by very large yaw velocities, a fifth threshold at 2000°/s was applied. Large isolated turns and turns that 'ride' on the slope of another turn may be detected repeatedly by different thresholds and thus may be counted several times. By omitting the redundant turns that have the same peak yaw velocity at the same point in time these identical turning events were only counted once.

The fly's pitch angle could not be determined for the entire flight (see above). Because the pitch was not analysed further in this study, the results obtained for the pitch angles during flight are placed in this section. At the beginning of most chases, many flies started pursuing from relatively far below the target (mean vertical distance between fly and target: 100 mm; std=23mm, n=10), and the animals exhibited a large pitch angle (mean=61°, std=11°, n=10). Therefore, at the beginning of most chases the target was seen against the ceiling of the arena. During the pursuit, however, the chasing male approached the target. The average vertical distance between fly and target decreased, measured at the middle of the chase it amounts to about 55mm (std=8.8mm, n=10). Additionally, the pitch decreased (mean=35°, std=11°, n=10), therefore the pursuer viewed the target in front of the grating pattern. This was also the case shortly before the end of the chase (mean distance=16mm, std=2.9mm, mean pitch = 27°, std=7°, n=10).

3.3 RESULTS

Male blowflies (*Lucilia sp.*) freely flying in a flight arena either were filmed from below and from the side with two digital high-speed cameras or from below only using a conventional video camera. The arena was surrounded by a stationary or moving vertically striped grating pattern. A small black sphere could be moved in the upper part of the flight arena to serve as a target for chasing behaviour. Depending on the visual environmental context, the flies executed three different flight behaviours: chases, cruising flights and optomotor flights. The characteristics and differences of these flight behaviours and the underlying control systems are analysed. Any turn of the chasing fly towards the moving target inevitably leads to retinal wide-field motion in the opposite direction. Since this global motion constitutes a powerful stimulus to the optomotor system, this eventually might cause the fly to execute compensatory turns, i.e. away from the target. Therefore, the question arises whether there is some form of interaction between the chasing control system and the optomotor control system.

For this reason, I test a possible impact of large-field visual motion on the performance of chasing behaviour. On the one hand, I characterise chasing behaviour with respect to the frequency of its success under different background conditions. On the other hand, the flight trajectories of chases recorded under different background conditions were reconstructed in 3D-coordinates and investigated with respect to various flight parameters, such as yaw velocity, forward velocity, turning frequency, retinal error angle of the target and distance between fly and target.

The flight behaviours

Chasing flights

The chasing behaviour (Fig. 3.4A) in male flies is assumed to be guided by a control system that presumably exists in males only (Collett, 1980a; Collett and Land, 1975; Collett and Land, 1978; Land 1993a; Land, 1993b; Land and Collett, 1974; Wagner, 1986a; Wagner, 1986b; Wagner, 1986c; Wehrhahn, 1979; Wehrhahn et al., 1982; Zeil, 1983). In my experimental set-up a black sphere served as 'female' dummy that moved with constant velocity (about 700°/s) on a circular track. This target was pursued by the male flies from below and behind. During chasing, the male frequently flies slightly outside the circular track of the target (Fig. 3.4Ai). According to a previous study, I classified the chases by their catching success (Boeddeker et al., 2003): In successful chases the target was caught (hence their name: 'C-chases') after short time (mean duration=371ms, std=172ms, n=50). After the target had been captured, the male fly may remain thereon up to several laps. In unsuccessful chases, the target was pursued ('P-chases') for at least one lap of the target (510 ms) without catching it. The fly either approached but missed the target,

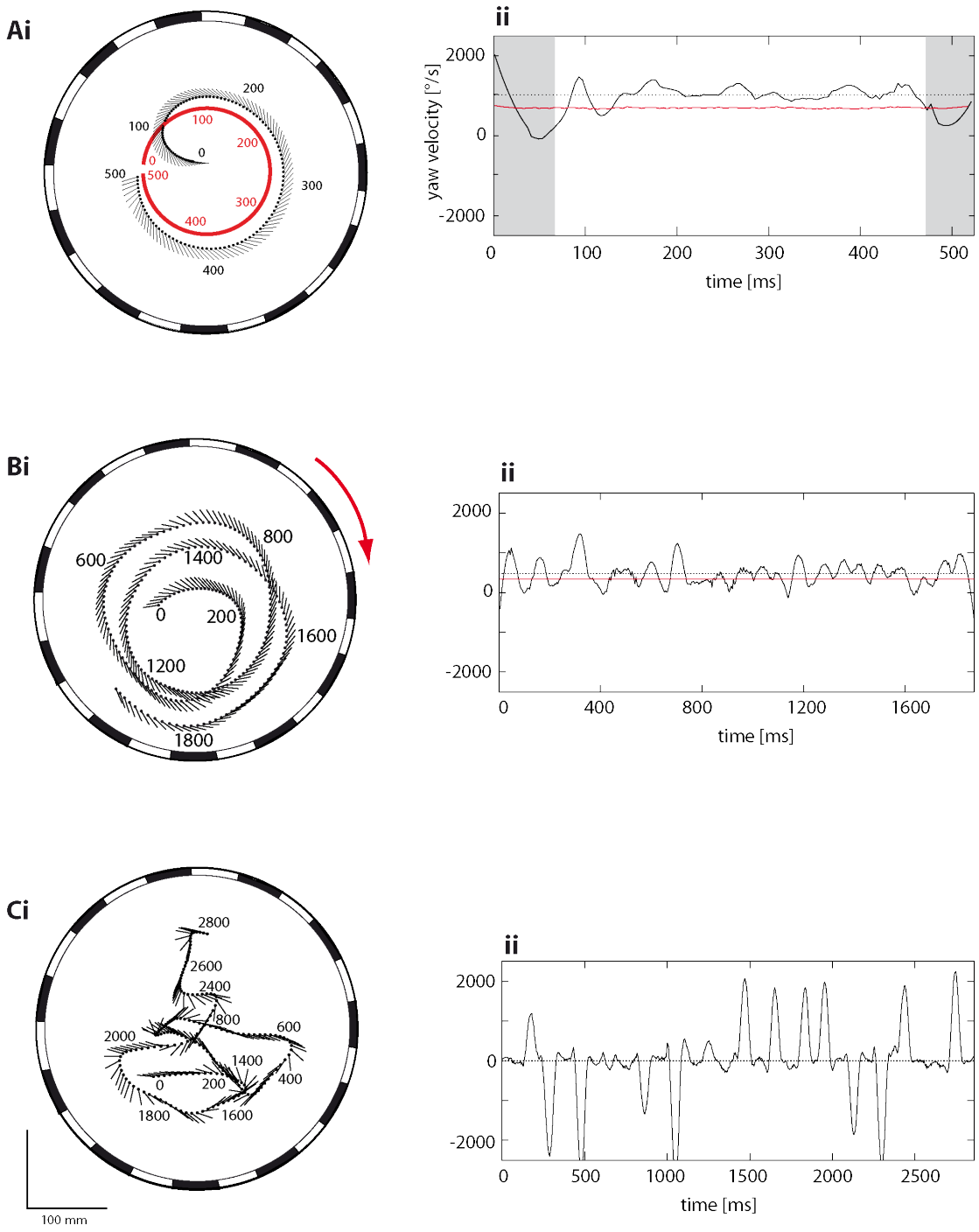
or it gave up chasing and retired. Figure 3.4Aii depicts the angular velocity of the fly's body about the yaw axis during a chase in front of a stationary background (i.e. the grating covering the walls of the flight arena was not moving). While pursuing the target, the mean angular velocity of the fly is close to the target's speed (Fig. 3.4Aii). During the chase the fly continuously changes its angular (i.e. yaw) orientation and performs smooth body turns of varying amplitude. Turns are defined as discrete events by thresholding (for the definition of turns and for details of thresholding see chapter 3.2). The chasing behaviour has been modelled and, despite these yaw velocity fluctuations, it has been concluded to be mediated by a smooth control system (Boeddeker and Egelhaaf, 2003; Boeddeker and Egelhaaf, 2005). This model of the chasing controller was concluded to use the retinal error angle of the target as relevant input. The error angle describes the fixation error of the target, i.e. the deviation of the target from the frontal position of the retina of the chasing male. The model of the chasing control system converts the retinal error angle by a continuous transformation into smooth turning reactions. Much larger turning velocities are observed mainly at the beginning of chases when the fly makes an initial turn towards the target and at the end of the chase when the fly tends to orient itself almost orthogonally to the target's direction of movement (Boeddeker et al., 2003).

Optomotor response flights

To elucidate the impact of a moving wide-field pattern on the behaviour of free-flying blowflies, they were filmed in a moving environment. Therefore, the vertical grating on the walls of the flight arena was rotated horizontally at a constant velocity either slowly (45°/s) or fast (365°/s). The flies followed the visual wide-field motion on a roughly circular track by continuous body rotations. By these body turns of varying amplitude the flies tend to reduce the retinal slip velocity induced by the imposed wide-field motion (Fig. 3.4Bi). This so-called optomotor following response and the underlying control system have been examined extensively in tethered flight throughout the last decades (Götz, 1975; reviews: Egelhaaf and Borst, 1993; Hengstenberg, 1993; Heisenberg and Wolf, 1984; Reichert, 1993). Optomotor following responses are assumed to compensate for asymmetries in the animal's sensory and motor systems during locomotion (reviews: Collett et al., 1993; Wehner, 1981). Because the fly follows more or less closely the movements of the background, the mean yaw velocity of the fly is close to the respective angular velocity of the background (Fig. 3.4Bii).

Cruising flights

The term cruising flight is used here for flights which do neither have an obvious goal (such as chases), nor constitute a following response. The dynamical features of cruising flights differ much from those of chases and optomotor flights. Figure 3.4Ci shows an example of a cruising flight of a freely flying male *Lucilia* in a stationary environment (i.e. stationary background). The flight path is quite jerky; the fly



performs a series of rapid changes in the orientation of its body long axis. The angular velocities generated during these rapid turns are much larger when compared to those generated during chases and optomotor flights (Fig. 3.4Cii). These rapid body turns of high angular velocities as exhibited during cruising flight are called – by analogy to rapid human eye movements – saccades (Collett and Land, 1975; Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999). Between saccades, i.e. during the so-called inter-saccadic intervals, the orientation of the fly's body long axis remains relatively stable. The profile of the fly's yaw velocity reflects

Figure 3.4 Flight trajectories of male flies, as seen from below, and time-dependent yaw velocities of **(A)** a chasing, **(B)** an optomotor and **(C)** a cruising flight. **(Ai)** Flight trajectory of a fly (*black*) chasing the target (*red*) which moved on a circular track. The position (*centroid*) of the fly is depicted by a *dot*, the orientation of its body long axis is depicted by the *line*. *Numbers* denote time (in ms) with respect to the start of the trajectory. The flight arena is surrounded by a stationary vertical grating. **(Aii)** The yaw velocity of the fly changes continuously and relatively slowly while pursuing the target which moves on a circular path at 700°/s (*red line*). The mean yaw velocity of the fly is near 1000°/s (*black dotted line*). The initial turn and the final turn (shaded regions) were excluded from the quantitative analysis of flight parameters (see Methods). Data of fly and target are plotted every 4 ms. **(Bi)** Flight trajectory of a fly performing optomotor responses to a vertical grating moving at 365°/s. The motion direction of the grating (i.e. the background) is indicated by the *red arrow*. The *numbers* denote the time in ms relative to the beginning of the displayed flight sequence. Data of fly and target are plotted every 8 ms. Same plotting conventions as in Ai. **(Bii)** The yaw velocity of the fly fluctuates around its mean of 528°/s (*black dotted line*). The average yaw velocity of the fly is somewhat larger than the velocity of the background (365°/s; *red line*). **(Ci)** Flight trajectory of a male fly cruising in the arena that is surrounded by a stationary background. The male exhibits the fly-typical saccadic flight style: fast rotational turns are intermitted by intervals of straight flight. Data of fly and target are plotted every 16 ms. Same plotting conventions as in Ai. **(Cii)** The yaw velocity profile of the fly reflects the saccadic flight style. Between the turns exceeding 2500°/s are time intervals of little or no rotation, hence the yaw velocity is near 0°/s. The *black dotted line* depicts the average fly's yaw velocity at 48°/s. Scale bars: 100 mm. Note the different x-axis scaling in Aii-Cii.

this saccadic flight style: Rapid turns in both directions with angular velocities of frequently over 2500 °/s are intermitted with periods of little or no rotation. This flight style is characteristic of freely cruising flies (Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999; review: Land and Collett, 1997).

Characteristics of and differences between cruising, optomotor and chasing flights

It is generally agreed that during cruising the flight path is to be stabilised by the optomotor system. The mode of operation of the optomotor system is well apparent by the optomotor responses to wide-field motion (Fig. 3.4B). By contrast, during chasing of a moving target, the flight behaviour is assumed to be guided by the chasing control system. What are the characteristic properties of these different flight behaviours and the underlying control systems? Are there differences between them and how can the differences be quantified?

To answer these questions I analysed quantitatively the flight trajectories of male blowflies that exhibited these different flight behaviours. Chases and cruising flights were recorded in front of a stationary vertical grating to simulate a 'normal' stationary environment. Moreover, optomotor flights were recorded while the background moved horizontally around the flight arena. Two different motion velocities were employed, that are slow (45°/s) or fast (365°/s) background motion. As distinguishing features of the different flight modes I analysed the average yaw velocity, the peak yaw velocity, the forward velocity as well as the turning

frequency. Moreover, to assess the smoothness of the individual time-dependent velocity profiles, I employed the standard deviation of the velocity fluctuations around the time-averaged velocity profile. Additionally, I employed the average peak yaw velocities attained during the flights. The peak velocity is given by the maximum yaw velocity measured during each turn. Note that the here presented average peak yaw velocity values do not denote absolute values. Rather, they represent the relative differences with respect to the velocity profile: The average peak yaw velocity values were determined by averaging from the time-dependent velocity profiles by measuring the maximum velocity of each turn after subtraction of the mean yaw velocity from the velocity profile.

Yaw velocity

Both the mean yaw velocity and mean the peak yaw velocity strongly reflect the flies' flight mode that depends on the actual background conditions (Fig. 3.5A). While cruising, the average yaw velocity is low (i.e. close to 0°/s), although male blowflies perform saccadic turns of large peak velocities (Fig. 3.4Cii). The only small average yaw velocity results, on the one hand, from the straight flight sequences between saccadic turns since then the yaw velocity values are close to 0°/s, Fig. 3.4Cii. On the other hand, the saccadic turns are, as expected for cruising flights in a stationary environment, about equally distributed in both directions. Thus, positive and negative velocities may occur with an approximately equal share (see below).

In optomotor response flights the flies tried to follow the moving background. Therefore, the average yaw velocity is increased, i.e. 279°/s during slow, and 426°/s during fast background rotation (Fig. 3.5A). The fluctuations of the yaw velocity around the mean velocity value are much smaller in optomotor flights when the background moves than in cruising flights when the background is stationary (compare Fig. 3.4Bii with Fig. 3.4Cii). Moreover, the average peak yaw velocity is considerably lower during background motion (699°/s at slow and 608°/s at fast background motion) than in a stationary environment (about 1250 °/s), (Fig. 3.5A). Altogether, the trajectories of optomotor flights are smoother than those of cruising flights.

During chasing, the males exhibit steady turns at high velocities. The average yaw velocity is close to the angular velocity of the target that moves at 700°/s. Only rarely the yaw velocity reaches 0°/s for a longer time. By contrast, in cruising flights the fly does not turn much between saccades. The average peak yaw velocity during chases is small when compared to the values in cruising flights, and it is roughly in the range of the values of optomotor flights (Fig. 3.5A). Hence, when pursuing a target that moves on a circular path and when following a continuously moving optomotor stimulus, the fly's yaw velocity profile is substantially smoother than during cruising flights.

As already mentioned above, there are pronounced turns at the beginning and at the end of most chasing manoeuvres that are essential parts of the chase. Because in my study the focus concentrates on the flies' chasing performance *during* pursuit, these large turns were not included into the quantitative analysis regarding the parameters yaw velocity, forward velocity, turning frequency and error angle.

Forward velocity

The mean forward velocity and its fluctuations are shown in Figure 3.5B for blowflies during cruising, optomotor and chasing flights. The average forward velocity during cruising in the 0.2m radius flight arena is 0.4m/s. This value increases during optomotor responses with increasing background velocity to 0.55m/s and 0.8m/s during slow and fast background motion, respectively. Note that in the latter situation the velocity is twice as large as during cruising flights. While pursuing a target moving with 1m/s (asterisk in Fig. 3.5B), the chasing fly has an average forward velocity of 1.18m/s. It is remarkable that during chasing, males can at least triplicate their 'normal' forward velocity, as exhibited during cruising flights in the same flight arena. Independent of the exact environmental conditions and the flight mode, the velocity fluctuations range on average between 0.11m/s and 0.18m/s around the mean forward velocity. Hence, at least during optomotor responses and during chasing flights the variations of forward velocities are small relative to the respective mean velocities.

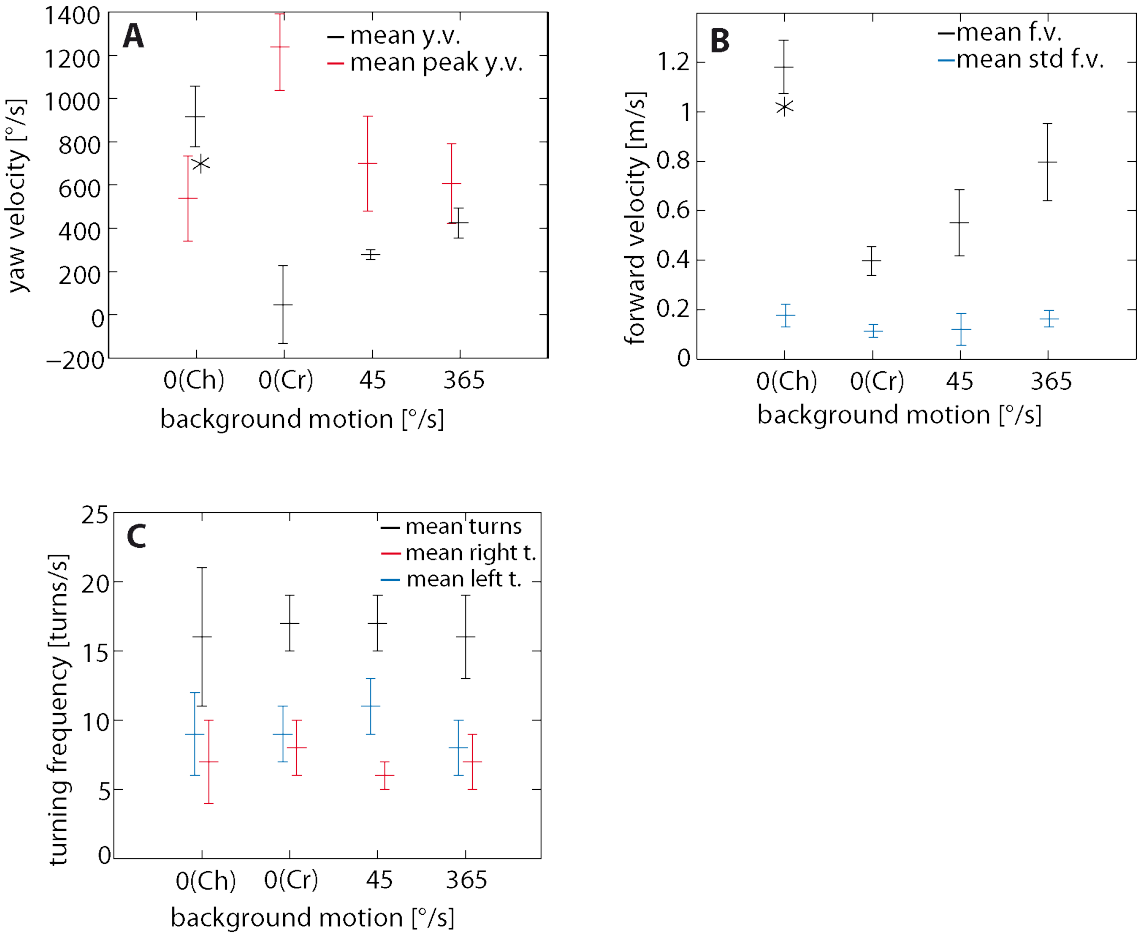
Turning frequency

Blowflies, on average, execute 16-17 ± 3 turns/s during cruising, optomotor and chasing flights indicating that the number of turns is quite independent of the flight behaviour and of the environmental condition (Fig. 3.5C). During these flights blowflies show a tendency to perform more left turns than right turns. Exclusively in chases, a bias in turning direction might be the consequence of the special experimental conditions, although this bias is expected to be opposite: While chasing, the visual stimulus is fixated in the frontal part of the visual field. When losing the stimulus, correcting turns may serve to bring the target back into the frontal field of view. To fixate the target, a clockwise turning target should thus require the chasing fly to perform more turns to the right than to the left. This expectation is not supported by the chases shown in Fig. 3.5C; however, this expectation is supported by the analysis of more chases (n=73; data shown below). These data are discussed below, since these chases were performed in front of moving backgrounds. In optomotor flights the bias in turning direction may be a consequence of the small amount of data. During cruising flights the turns are expected to be equally distributed in both directions, because no actual visual stimulus can bias the turning direction. Nevertheless, I find a slight (3 turns/s) bias toward turns to the left. However, this presumably may result from chance, as will be emphasized by results shown below.

To conclude the results so far, (1) the visual motion stimuli used in these experiments, i.e. the target and the vertically striped background, obviously are potent stimuli to reliably induce behavioural responses, i.e. chasing behaviour and optomotor following responses. (2) There are clear differences between the different flight modes, indicating that flies adjust distinct flight parameters (e.g. yaw- and forward velocity) strongly to the actual task and according to the actual visual stimulation.

Chasing behaviour during background motion

During chasing, when the target deviates from the frontal midline of the visual field, the chasing system is assumed to minimise this 'fixation error' by a command to turn towards the target (Boeddeker and Egelhaaf, 2003). Any turn of the fly towards a target inevitably leads to wide-field motion in the opposite direction on the fly's retina. This opponent global visual motion may activate the optomotor system that may induce a turn that is counterdirected to the global motion. This turning command of the optomotor system is as well countering the turning command of the chasing system. Consequently, in this situation, the two control systems may be in conflict with each other.



To elucidate the potential impact of the optomotor system on chasing behaviour, I conducted a set of further behavioural experiments with simultaneous presentation of a chasing stimulus and optomotor wide-field motion. These stimuli are on the one hand the dummy target moving at 700°/s on a circular track, and on the other hand the vertical grating that surrounded the flight arena (i.e. the background) moving in the same or in the opposite direction to the target. I analysed chasing behaviour under five different background conditions. Condition 1: the vertical grating was held stationary as a reference. Conditions 2-5: the grating either moved slowly (45°/s) or fast (365°/s) in the same direction as the target ('positive background motion') or in the opposite direction to the target ('negative background motion') (see also Methods). Hence, while chasing the target, the optomotor stimulation of the fly was either decreased (at positive background motion) or increased (at negative background motion) with respect to the normal stationary environment. I analysed the performance during P- and C-chases with respect to several flight parameters and investigated their dependence on the background motion.

Figure 3.5 **(A)** Yaw velocity, **(B)** forward velocity and **(C)** turning frequency during chases, optomotor and cruising flights. Chasing flights (left pair of data points in each figure; *Ch*) and cruising flights (second pair of data points; *Cr*) are performed in front of a stationary background (*0°/s*). Optomotor flights are displayed in front of a slowly (*45°/s*) and fast (*365°/s*) moving background (third and fourth pair of data points). Data obtained from chasing flights represent the averages over C- and P-chases. **(A)** Time averaged yaw velocities (±std) of a sample of flies (*mean y.v., black*) and mean peak yaw velocity (±std) of the same sample of flies (*mean peak y.v., red*) obtained from chasing, cruising and optomotor flights. The peak yaw velocity was determined from the time-dependent velocity profiles by measuring the peak velocity of each turn after subtraction of the mean yaw velocity from the velocity profile. The flies' mean yaw velocity is near 900°/s for males pursuing a target that moved around 700°/s (*asterisk*). In cruising flights, the mean yaw velocity is close to 0°/s. In optomotor flights the fly's mean yaw velocity increases with increasing background motion. **(B)** Time averaged forward velocity (±std) of a sample of flies (*mean f.v., black*) and the mean standard deviations (±std) of the corresponding velocity fluctuations (*mean std f.v., blue*). The flies' mean forward velocity is around 0.4m/s in a stationary environment during cruising flights (*Cr*); it increases with increasing background motion velocity (*45°/s* and *365°/s*). The fly's mean forward velocity is around 1.2m/s for flies chasing (*Ch*) the target moving at 1m/s (*asterisk*). **(C)** The average turning frequency (±std). (*mean turns, black*) is relatively similar for chases, optomotor flights and cruising flights. Red and blue markers indicate the average frequency (±std) of right (*mean right t., red*) and left (*mean left t., blue*) turns. Cruising flights: n=10, total flight time (TFT)=20804ms; optomotor flights at 45°/s: n=4, TFT=1820ms; optomotor flights at 365°/s: n=8, TFT=5544ms; chases: n=17, TFT=4740ms.

Catching success and flight motivation

Successful chasing behaviour may be terminated with catching the target. To detect a potential impact of optomotor stimulation on the chasing performance, I assessed whether the catching success is influenced by background motion. I counted the number of catches occurring within 250 independent 40s time-windows (Fig. 3.2A, see Methods). As can be seen in Figure 3.6A, the flies on average performed between 14 and 17 catches when the background was stationary or moved slowly in either direction. Even at high background velocities male flies were still able to catch their target frequently. However, the catching frequency decreases by about one third to below 10 catches per time-window.

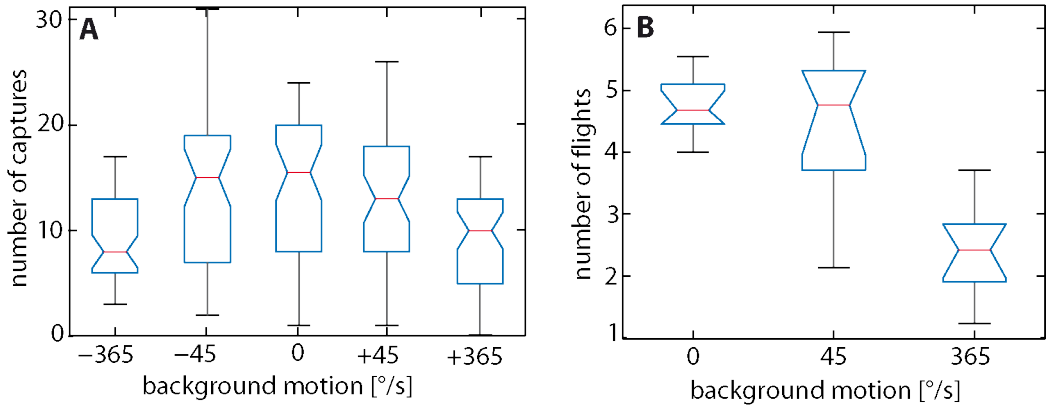


Figure 3.6 Box-Whisker plots of the number of captures of the target and the number of flights dependent on background condition. **(A)** The number of captures was counted for male blowflies chasing a target that moved at 700°/s. The captures were counted in 50 time-windows (each lasting 40s) that were each recorded at five different background conditions, resulting in overall 250 analysed time-windows. The background was either moving slowly (*±45°/s*) or quickly (*±365°/s*) in the same (*positive*) direction as or in the opposite (*negative*) direction to the target. As a reference, the background was held stationary (*0°/s*). The catching frequency is significantly decreased at high background velocities with reference to the number of captures obtained while the background was stationary (Wilcoxon signed rank test; $P<0.01$). Captures: n=2954. **(B)** Number of flies flying in the arena in front of three different backgrounds. The background was either stationary (*0°/s*, reference), or moved at *45°/s* or at *365°/s*. The flight frequency was counted during each 1s time bin for a 30s time-window and averaged over the 30s time-window. The averages shown in the figure are obtained from 10 trials. The number of flights was found to be significantly smaller at the high background velocity with reference to the stationary background (Kruskal Wallis Test; $P<0.01$). 1s time-bins: n=1800. (For a detailed explanation of the experimental procedure see Fig. 3.2)

One potential reason for this decrement may be a reduction in the overall number of flights when background motion is fast. I thus counted the number of flies flying in the arena within 30s time-windows during stationary, slow and fast background motion respectively. As can be seen in Figure 3.6B, the number of flies that actually started to fly around is similar in the arena with a stationary or a slowly moving background, but is by about one half decreased when the background pattern moved

fast. Hence, fast background motion obviously diminishes flight motivation, and this might explain the corresponding decrease of catching frequency. These results suggest that the catching success does not deteriorate as a consequence of background motion after the flies initiate a chase.

Nevertheless, these data do not exclude that background motion influences the flight behaviour during the chase in a more subtle way. To quantify a possible impact of optomotor stimulation on the fine structure of the chasing flights, I performed a more detailed analysis by evaluating the temporal structure of the chasing trajectories.

Yaw velocity and peak yaw velocity

Since it cannot be excluded that the impact of optomotor stimulation is different for C- and P-chases, the corresponding trajectories were analysed separately. The average yaw velocity of male blowflies during C- and P-chases and for all background conditions amounts to between 800°/s and 900°/s (Figs. 3.7A,B). Because the chasing fly follows the target that moves at 700°/s, the fly's yaw velocity is on average close to the angular velocity of the target. The results for the C- and P-chases are generally similar, although P-chases are less variable than C-chases. For both, C- and P-chases and for the five different background conditions, there is no consistent effect of background motion on the fly's angular velocities. Similarly, I find no consistent impact of background motion on the average peak yaw velocities (Figs. 3.7A,B). Moreover, the average peak yaw velocities in C- and P-chases and for all background conditions are relatively small (between 400°/s and 800°/s). (Remember that these average peak yaw velocity values do not denote absolute values, but relative differences with respect to the velocity profile.)

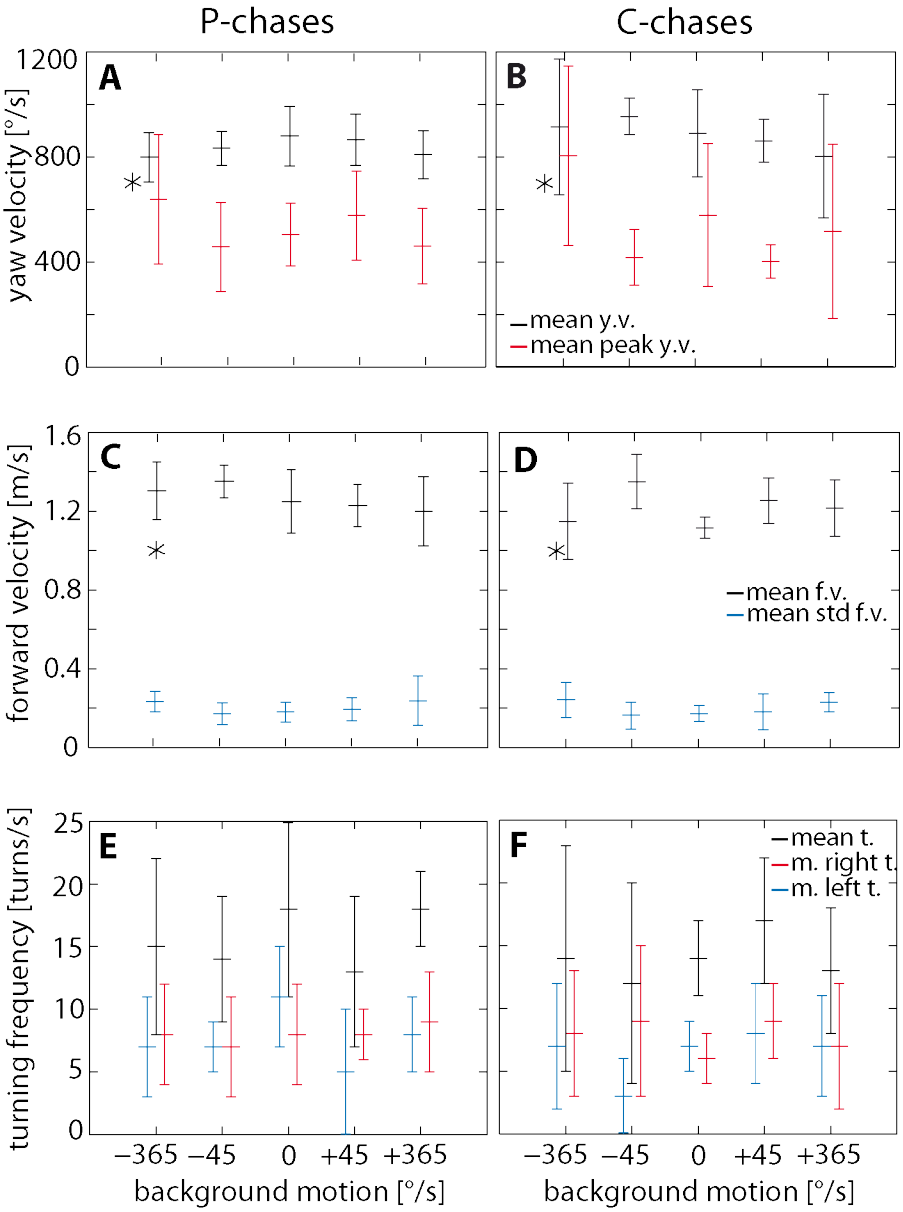
Analysis of forward velocity, turning frequency, distance and error angle

Because in the present experimental setup male flies pursued a dummy moving at 1m/s, the flies' average forward velocity is close to 1m/s (Figs. 3.7C,D). When flying a similar trajectory as the target (cf. Fig. 3.4Ai), the pursuer has to fly faster than the target to reach and catch it. In C- and P-chases the average forward velocities range between 1.12m/s and 1.35m/s. Again, there is no consistent effect of optomotor stimulation on the forward velocity for both C- and P-chases. In both chasing 'modes', the velocity fluctuations are small (on average around 0.2m/s) and increase only slightly with increasing background velocity.

The turning frequency ranges in C- and P-chases for all background conditions between 12 and 18 turns per second (Figs. 3.7E,F). There is a slight decrease of turning frequency for slow pattern motion in P-chases, but not in C-chases. Hence, also the turning frequency is not consistently influenced by background motion. In C- and P-chases the flies execute, on average, more turns to the right than to the left. As mentioned above, the pursuit of the clockwise moving target might require

more rightwards turns to bring back the target into the frontal visual field. However, these differences in the number or left and right turns are very small and statistically insignificant (Wilcoxon signed rank test, P=0.49).

As a potential indicator of effects of background motion on chasing behaviour I assessed in C-chases the time course of the flies' distance to the target for the last 200ms of the chase. Since C-chases are terminated with catching the target, the flight trajectories have a distinct end point. In contrast, in unsuccessful P-chases the fly either misses the target during the final turn or gives up chasing. Because P-chase trajectories do not exhibit a clear endpoint of the chase, I evaluated the distance only in C-chase trajectories. One might expect that a chasing fly could be retarded by negative background motion and pushed towards the target by positive



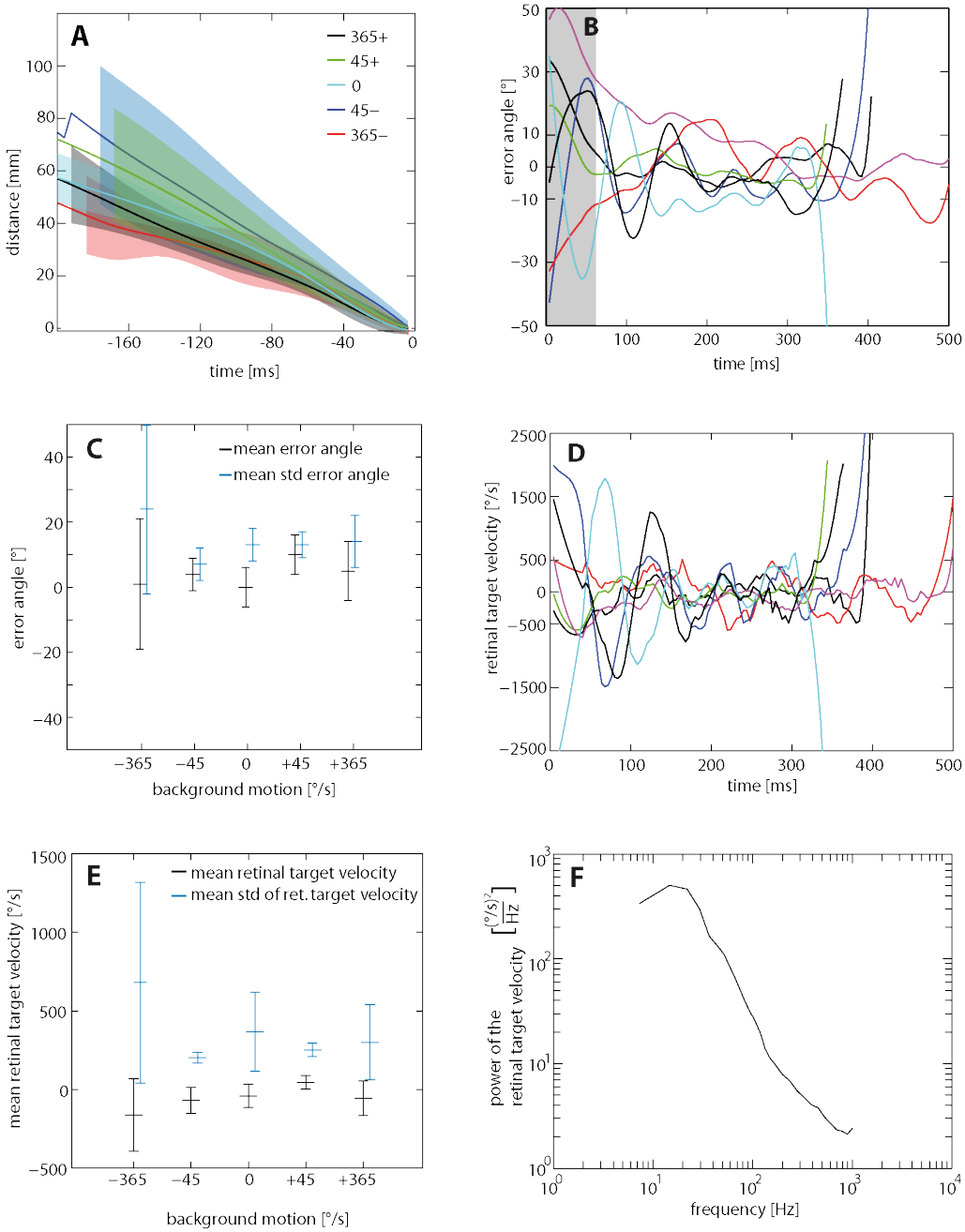
background motion, respectively. Although there is some variability in the time course of distance reduction with background velocity, no consistent background velocity dependence is observed (Fig. 3.8A). Lastly, the standard errors of the averages widely overlap, which further supports the conclusion that the flies' distance to the target is not affected by background motion.

Finally, I investigated whether background motion has an impact on the error angle of the target on the pursuer's eye. Since P-chases are defined by the pursuer giving up chasing or by missing the target, the error angle of a whole chase is evaluated only for C-chases. Examples of the time course of the error angle during different chasing flights are shown in Fig. 3.8B. With the exception of the initial turn (executed mainly within the first 60 ms of each chase, grey area in Fig. 3.8B) and the final turn (executed at the end of each chase, Fig. 3.8B), the error angle is, on average, between 0° and +10° (Fig. 3.8C). This finding indicates that the target is well fixated within the frontal eye region. Furthermore, the average fixation on one side of the retina between (0° and +10°) indicates that during the clockwise pursuits the target position is slightly shifted on the retina in the direction in which it would move on the eye if it were not fixated.

Does background motion have an impact on the target's error angle during a blowfly's chase? There is a slight increase in the fixation error at slow background velocities. However, this tendency is no longer obvious at high background velocities (Fig. 3.8C). The fluctuations of the error angle are, on average, somewhat larger at high background velocities. These values range between 7°-24°. This indicates that the target is still fixated within the frontal part of the visual field which can be seen by the time course of the error angle during chases (Fig. 3.8B). This part of the visual field can be considered to be frontal, since flies have, due to

Figure 3.7 Yaw velocity, forward velocity and turning frequency determined separately for C- and P-chases for five different background conditions. Condition 1: the grating was held stationary as a reference (*0°/s*). Conditions 2-5: the grating either moved slowly (*45°/s*) or fast (*365°/s*) in the same direction as the target (*+45°/s* or *+365°/s*) or in the opposite direction to the target (*-45°/s* or *-365°/s*) (see also Methods). **(A, B)** Time-averaged (±std) yaw velocity (*mean y.v., black*) and mean (±std) peak yaw velocity (*mean peak y.v., red*) determined for P-chases **(A)** and C-chases **(B)**. The target moved at 700°/s (*asterisk*). The peak yaw velocities values were determined in the same way as described in Fig. 3.5A. **(C, D)** Time-averaged (±std) forward velocities (*mean f.v., black*) and the average (±std) velocity fluctuations (*mean std f.v., blue*) determined for P-chases **(C)** and C-chases **(D)**. The flies followed the target that moved at 1m/s (*asterisk*). **(E, F)** Mean (±std) frequency of right (*m. right t., red*) as well as left (*m. left t., blue*) turns, and overall average (±std) turning frequency (*mean t., black*) determined for P-chases **(E)** and C-chases **(F)**. Mean and standard deviation were calculated across flies. Cond. 1 (0°/s): C-chases n=7, TFT=1896ms; P-chases n=10, TFT=2844ms. Cond. 2 (+45°/s): C-chases n=7, TFT=2288ms; P-chases n= 8, TFT=2492ms. Cond. 3 (-45°/s): C-chases n=4, TFT=748ms; P-chases n=8, TFT=3912ms. Cond. 4 (+365°/s): C-chases n=9, TFT=2788ms; P-chases n=7, TFT=3108ms. Cond. 5 (-365°/s): C-chases n=9, total flight time (TFT)=2928ms; P-chases n=9, TFT=4024ms.

their large facet eyes, an almost 360° panorama view, which has been supported by the examinations of the receptive-field organisation of optic flow processing neurons (e.g. Krapp et al., 1998). Altogether, I find no indications of any systematic dependency of the fixation performance on different background velocities. Furthermore, I analysed the velocity at which the target fixation error is varied on the retina of the chasing male. The time course of the retinal target velocity during seven different chasing flights is depicted in Fig. 3.8D. The analysis reveals that on average the retinal target velocity is around 100°/s, and the amplitude of the velocity fluctuations ranges between 200°/s and 600°/s (Fig. 3.8E). The power spectrum of the error angle velocity indicates that frequencies of up to approximately 30-50Hz are prominent in the time-dependent retinal target velocity (Fig. 3.8F).

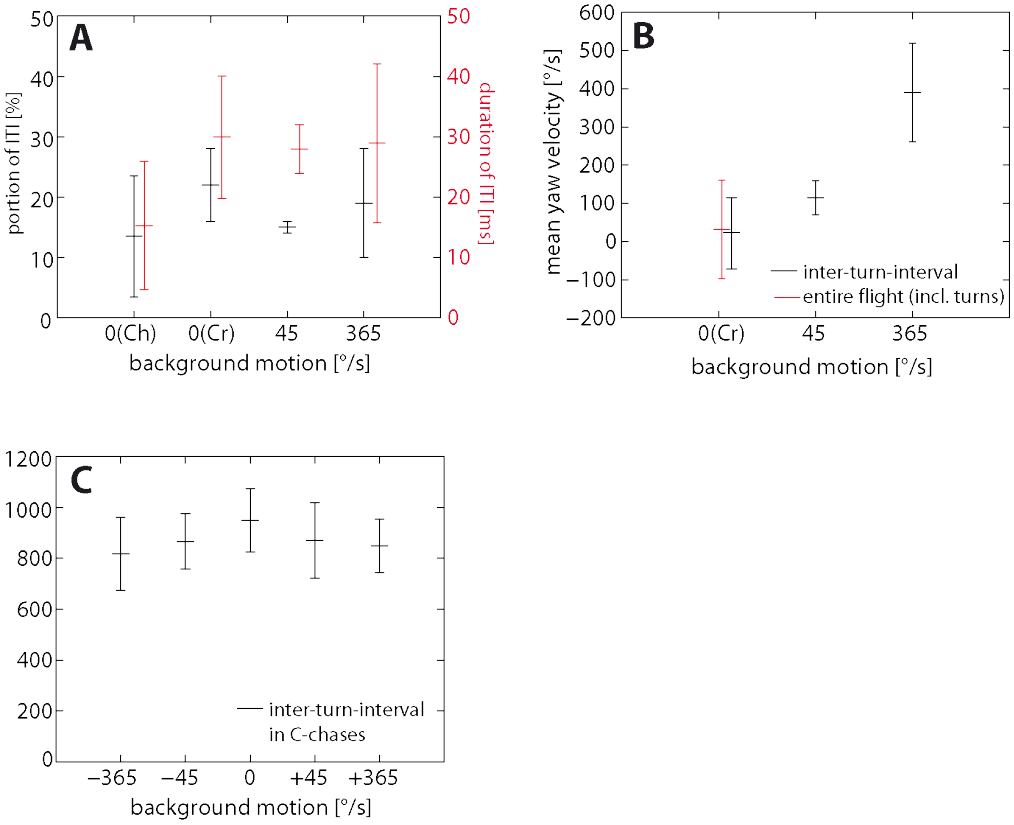


Analysis of the intervals between turns

Blowflies smoothly change their body yaw orientation during chasing a target that moves on a circular track as well as during exhibiting the optomotor response to a steadily moving background. The velocity profiles of the turns exhibited during chasing and optomotor behaviour differ in three aspects from the rapid turns (saccades) performed during cruising flights. First, the average peak yaw velocities that are reached during saccades in cruising flights are about twice as large (around 1300°/s) than those reached during chasing (around 550°/s) and optomotor flight (between 600°/s-700°/s; Fig. 3.5A). Second, the duration of the time intervals between the body turns and their proportion relative to the entire flight differ within the three flight behaviours. In cruising flights, the proportion of these inter-turn intervals relative to the entire flight is about 20%. This value is slightly decreased during optomotor flights and is clearly decreased during chases (Fig. 3.9A). Accordingly, the mean duration of the intervals is 30ms in cruising flights; it is slightly shorter in optomotor flights and is as small as 15ms in chases (Fig. 3.9A).

Figure 3.8 Distance between chasing fly and target, error angle of the target and retinal velocity of the target determined for C-chases in dependence on the background conditions. **(A)** Mean time course of the flies' distance to the target for different background velocities. The time course of the distance is shown for the last 200ms before capture for C-chases. The data were aligned according to the end of the flight episode (i.e. the catch represents time 0ms). *Coloured lines* represent the mean time course, similarly *coloured shaded areas* depict the respective standard error of the mean. For the sake of clarity, these areas are shortened differentially. Mean and standard deviation were calculated across flies. The analysis was based on the same C-Chases as described in Fig. 3.7.: n=36, total flight time=7200ms. **(B)** Time dependent deviation of the retinal position of the target from the frontal midline of the chasing fly ('error angle') of seven chasing flights. At the beginning of each chase, mainly within the first 60ms, the large error angle (*grey area*) is compensated by an initial turn that centres the target within the frontal eye region. The scaling of the y-axis includes the eye region of increased acuity (i.e. acute zone) in male flies (Land and Eckert, 1985). At the end of the chase the fly turns towards the target to grab it with its legs. During this final turn, the target moves out of the frontal eye region. During the chase, the target is fixated mainly within the frontal eye region. Chases: n=7; total flight time=1896ms. **(C)** For the calculation of the mean (±std) error angle over time (*mean error angle, black*), the initial and the final turn were omitted from the analysis (for details see Methods). The mean error angle lies between 0° and 10°, the average (time-dependent) fluctuations of the target on the eyes (*mean error std angle, blue*) range between 7° and 24°. The scaling of the y-axis includes the eye region of the acute zone in male flies (Land and Eckert, 1985). Chases: n=36, total flight time=10648 ms. **(D)** Time course of the retinal target velocity of seven chasing flights. The same colours are used for the corresponding flights in (D) and (B). Chases: n=7; total flight time=1896ms. **(E)** Average (±std) retinal target velocity obtained from a sample of flies (*mean retinal target velocity, black*) flying in front of five different backgrounds and the average standard deviations of the velocity fluctuations around the mean (*mean std of retinal target velocity, blue*). The initial and the final turn of the chases were omitted from the analysis. Chases: n=36, total flight time=10648 ms. **(F)** To calculate the power spectrum of the retinal target velocity data of the chasing flights obtained under all background conditions were pooled. The initial turn and the final turn of the velocity data were omitted from the analysis. Chases: n=78; total flight time=27028ms.

Third, the yaw velocity measured in the inter-turn intervals differ within the three flight modes. During cruising, the flies fly relatively straight within inter-saccade intervals, which means that they perform little or no rotation (Fig. 3.4C; see also Kern et al., 2005; Kern et al., 2006; Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999; Wagner, 1986b; Wagner, 1986c). Thus, the mean yaw velocity within inter-saccade intervals is around 0°/s (Fig. 3.9B). Furthermore, since the saccadic turns are about equally distributed into both directions, the mean yaw velocity as measured over the entire cruising flight including the turns is as well around 0°/s (Fig. 3.9B). In optomotor flights the mean yaw velocity measured from inter-turn intervals is much larger than 0°/s and increases with increasing background velocity (Fig. 3.9B). In chasing flights the mean yaw velocity measured from inter-turn intervals is around 940°/s, quite independent from the actual background velocity (Fig. 3.9C). This indicates that during both chases and optomotor flights the flies follow the respective visual stimulus by mainly smoothly changing their body yaw orientation and thus generate more or less continuous rotations within the inter-turn intervals. Yet, chases and optomotor flights are not absolutely smooth: around the more or less continuous rotations the flies generate body turns that are small compared to saccades as exhibited during cruising flights (Figs. 3.4Aii, Bii, Cii). In any case, these smooth flight patterns are in stark contrast to the saccadic flight style that is characteristic for cruising flights. Altogether, these results infer that both chasing and optomotor response are smooth in nature in contrast to the saccadic cruising behaviour.



Are there sex-specific differences during cruising flights?

So far, the experiments of the present study have exclusively been performed with male flies. In contrast, the majority of previous studies on flight behaviour in flies were conducted with females. To be able to compare the present results with those of previous studies, I wanted to investigate potential sex-specific differences in the flight pattern during cruising behaviour. This was done by analysing the flight trajectories of male and female blowflies while cruising in the flight arena in front of a stationary background. Males and females in these experiments were of about the same age.

Male and female blowflies exhibited the fly-typical saccadic flight style. Both sexes perform rapid saccadic turns at about the same frequency (Fig. 3.10A) that were separated from each other by periods of little or no rotation (see Fig. 3.4C for an example in males). In both sexes, these saccadic turns are about equally distributed in both directions since the turning direction does not differ significantly between females and males (Wilcoxon sign-rank test; P=0.05). Therefore, the average yaw velocity in both sexes is close to 0°/s (Fig. 3.10B). In contrast, the average peak yaw velocities that are reached during the rapid turns differ significantly between females (900°/s) and males (1250°/s) (Wilcoxon sign-rank test; P<0.01). Blowflies of both sexes displayed, on average, similar forward velocities (Fig. 3.10C). Moreover, the forward velocity fluctuations are small and again similar in both sexes (average values of 0.13 m/s for males and of 0.12 m/s for females). These results show that at least in the flight arena used in my study the overall characteristics of cruising flights of blowflies is similar in both sexes, except that under the conditions in the here used experimental setup male blowflies execute saccades with larger amplitudes than females.

Figure 3.9 Time intervals between turns during cruising, optomotor and chasing flights. **(A)** Average (±std) percentage of the inter-turn interval (*ITI, black*) of the entire flight of each fly and mean (±std) duration of the inter-turn intervals (*ITI, red*) in ms. The percentage denotes the fraction of inter-turn intervals of each flight. Mean and standard deviations were obtained by calculating the portion as well as the duration of inter-turn intervals of each flight averaged across flies. Chasing flights (left pair of data points in each figure; *Ch*) and cruising flights (second pair of data points; *Cr*) were performed in front of a stationary grating (*0°/s*). Optomotor flights (third and fourth pairs of data points) were displayed in front of a slowly (*45°/s*) and fast (*365°/s*) moving background. Data of chasing flights represent the average across C- and P-chases. **(B)** Time-averaged (±std) yaw velocity during the inter-turn intervals (*black*) of cruising and optomotor flights of each flight averaged across flies. Time-averaged (±std) yaw velocity over the entire flight (*red*) (including turns) of cruising flights averaged across flies. **(C)** Time-averaged (±std) yaw velocity during the inter-turn intervals of C-chases performed under five different background conditions. (Same background conditions as in Fig. 3.7). The mean and standard deviation were calculated for each flight and averaged across flies. Cruising flights: n=10, total flight time (TFT)=20804ms; Optomotor flights at 45°/s: n=4, TFT=1820ms; Optomotor flights at 365°/s: n= 8, TFT=5544ms; Chases: n=17, TFT=4740ms.

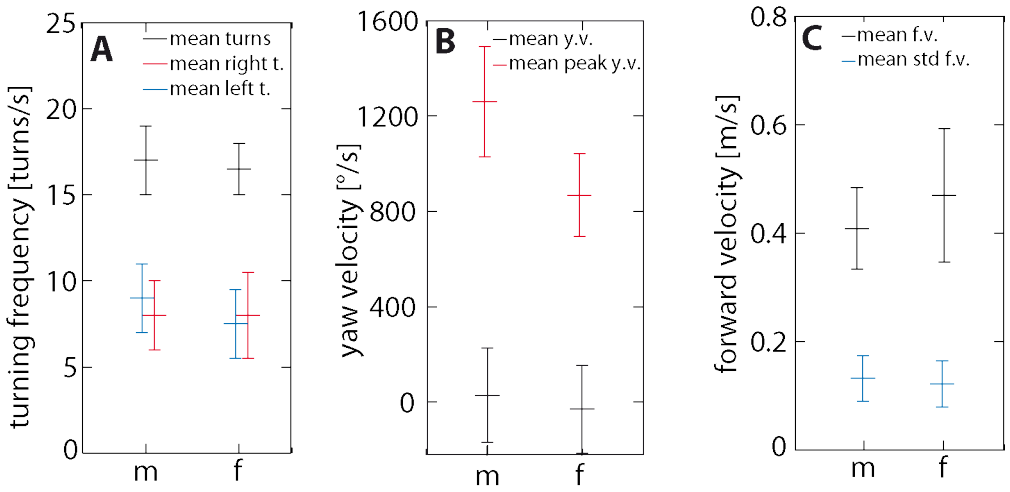


Figure 3.10 Turning frequency, yaw velocity and forward velocity during cruising flights of male (*m*) and female (*f*) blowflies. The animals flew in the flight arena in front of a stationary background. **(A)** Mean (±std) overall turning frequency (*mean turns, black*), as well as average (±std) frequency of right (*mean right t., red*) and left (*mean left t., blue*) turns are relatively similar in male and female blowflies. **(B)** Whereas mean (±std) peak yaw velocities (*mean peak y.v., red*) differ significantly (Wilcoxon sign-rank test; P<0.01) between males and females, the time-averaged (±std) yaw velocities (*mean y.v., black*) are relatively similar in both sexes. **(C)** Time-averaged (±std) forward velocities (*mean f.v., black*) and the mean (±std) standard deviations of velocity fluctuations (*mean std f.v., blue*) do not differ significantly in both sexes (Wilcoxon sign-rank test; P=0.05). Mean and standard deviation were calculated across flies. Females: n=10, total flight time=22740ms; Males: n=10, total flight time=20804ms.

3.4 DISCUSSION

In behavioural experiments I examined two flight control systems, the chasing control system of male flies and the optomotor control system which is implemented in both sexes. These two control systems steer the flight motor in different ways leading to different flight behaviours. Chasing behaviour is performed in the context of mating behaviour where male flies follow conspecifics, such as females. Optomotor behaviour refers to following responses elicited by coherent wide-field motion and is assumed to serve course stabilisation. Cruising flights, i.e. spontaneous flights that do not serve any obvious purpose will serve as reference for these two visually driven behavioural components.

The analysis of the flight trajectories indicate that the chasing behaviour in blowflies is guided by a smooth chasing control system, which is in accordance with earlier studies (Boeddeker et al., 2003; Boeddeker and Egelhaaf, 2005). Also the optomotor following responses are found to be guided by a smooth control system. In this regard they can clearly be distinguished from cruising flights where flies generally change flight direction not smoothly but by brief and rapid saccadic turns (Schilstra

and van Hateren, 1999). Because saccade-like and smooth pursuit strategies differ so much in their performance, they might be mediated by separate control systems (Land, 1992; Land, 1993b).

On the basis of both behavioural experiments and a detailed analysis of the resulting flight trajectories I investigated the two systems controlling chasing and optomotor behaviour not only in isolation, but also their interaction. The results of experiments employing optomotor and chasing stimuli simultaneously show that coherent large-field motion does not have a consistent impact on the chasing performance, indicating that during chasing, the gain is high for the chasing controller and low for the optomotor controller.

Finally I will discuss several models that previously have been proposed as possible explanations of the interaction between the control systems mediating pursuit and the optomotor following responses, respectively. I will reason from the behavioural results of my study to the nature of interaction of the chasing system and of the optomotor system. The visuomotor control systems in flies are in some aspects analogous to the control systems that guide eye movements in primates. Thus, my results on visual pursuit and on the integration of different control systems of visually guided behaviour will be discussed not only in the context of studies done on other insects but also in the context of vertebrate eye movements.

Methodological limitations

In my study I draw conclusions about the visually guided control systems of blowflies by analysing the flies' flight behaviour recorded with high-speed video cameras. On the image frames I could determine the position and the orientation of the body long axis of the flies. However, since the area of the flight arena that had to be spanned by the cameras had to be sufficiently large for the blowflies to show chasing and optomotor behaviour, it was not possible in most situations to resolve the fly's head orientation. This limitation may be critical since blowflies can move their head relative to their body during flight (Hengstenberg, 1993; Land, 1973; van Hateren and Schilstra, 1999). Rotations of the head relative to the surroundings about the pitch and roll axes are generally small during flight (Schilstra and van Hateren, 1998). Moreover, yaw rotations of the head are usually in phase, though somewhat faster than yaw body rotations (Kern et al., 2006; van Hateren and Schilstra, 1999). Therefore, I assumed in my analysis, as a first approximation, that the yaw angle of the head was aligned with the body long axis and that the roll and pitch angles of the head were held constant.

Most free-flight studies on visually guided orientation behaviour compute the optic flow information from the body yaw angle (e.g. Boeddeker et al., 2005; Collett, 1980a; Collett, 1980b; Olberg et al., 2000; Wagner, 1986a; Wagner, 1986b; Wagner, 1986c; Zeil, 1993) or even from the time course of the animal's position in space (Land and Collett, 1974; Zeil, 1986). There is only one series of electrophysiological

studies, so far, for which the optic flow was reconstructed from head orientation (Kern et al., 2005, Kern et al., 2006; Karmeier et al., 2006) which had been determined by an ingenious magnetic coil technique (van Hateren and Schilstra, 1999).

In my study, the error angle of the target relative to the frontal midline of the fly's visual field was calculated on the basis of the fly's body long axis by a line connecting the fly and the target's centre, which represents an appropriate approximation of the fixation error in the azimuth. If, while chasing, males had compensated to some extent the conflicting optomotor stimulation (see below) by yaw movements of the head, this might have affected the error angle. However, since head movements about the yaw axis are small during free flight (up to about 5°) (van Hateren and Schilstra, 1999), the error angle determined in my results would deviate from the real error angle by not more than 5 degrees. Even then would the fly fixate the target in the frontal visual field and the robust and reliable performance of the chasing control system would still be impressive.

Pursuit of a small target

When male blowflies encounter other flies or small targets (such as the black sphere used in my experiments) they will chase these with virtuosity and high accuracy. Due to its complex aerobatics the chasing behaviour of flies is one of the fastest visually guided behaviours that have been found in nature. How is visual information about the pursued object used by the pursuer? Generally, the chasing system is viewed as a feedback control system that minimises deviations of the images of small objects from the midline of the visual field. In blowflies, the chasing fly keeps the retinal position of the target in the frontal field of view by predominantly smooth rotations about the vertical body axis. Thus, the average error angle of the target on the retina is small during the chase (Fig. 3.8C; Boeddeker et al., 2003). During chasing, the flies' forward and the yaw velocities are adjusted to the target's flight dynamics. This means that the average forward velocities of flies that pursue a quickly moving target (700°/s in the present study) are increased trifold when compared to the forward velocities of flies performing cruising flight in the same flight arena (Fig. 3.5B). Similarly, the average yaw velocity is increased during chasing of the target (Fig. 3.5A). These two parameters, the forward and the yaw velocity, are assumed to be the effective motor outputs of the chasing control system: A recently published phenomenological model of the chasing controller uses the retinal size of the target as input to control the pursuer's forward velocity and the retinal position of the target to control the pursuer's angular velocity (Boeddeker and Egelhaaf, 2003; Boeddeker and Egelhaaf, 2005).

A control system that guides visual pursuit of a small target is not only a characteristic of male blowflies, but has been described in different insects, e.g. several species of flies (e.g. Boeddeker et al., 2003; Collett, 1980a; Land and Collett, 1974), praying mantids (Rossel, 1980), dragonflies (Olberg et al., 2000) and bees

(Gries and Koeniger, 1996), as well as in primates, including humans (reviews: Ilg, 1997; Land, 1992; Land, 1999). In insects the eyes are fixed within the head, therefore visual pursuit of a small object may be performed by head movements (e.g. praying mantis) or may include movements of the entire body. Since primates possess mobile eyes, visual pursuit may solely be accomplished by eye-movements without the need to move body and head.

Besides a chasing strategy, as found in blowflies, where the deviation of the retinal image of the target is transformed into a continuous turn, another strategy to pursue and catch a target becomes manifest in the pursuer flying on an interception course. Such a chasing strategy was characterized in hoverflies and dragonflies. Hoverflies (*Syrphidae*) are described to pursue conspecifics on a relatively straight flight path, which is determined by three input parameters: the target's position, velocity and acceleration (Collett and Land, 1978). The pursuit on an interception course is slightly different in dragonflies (*Libellulidae*): Whereas hoverflies direct their chases toward the currently perceived position of their target, dragonflies direct their flight paths to a point in front of the prey (Olberg et al., 2000). The prey is intercepted with a relatively straight flight trajectory. During pursuit, the dragonfly's head rotates relative to the rest of the body in order to stabilize the prey image on a specific eye region (Olberg et al., 2007).

Smooth or saccadic pursuit?

The smoothness of a flight trajectory is determined by the velocity with which flies execute body turns around the yaw axis. To determine the smoothness of chases, I need to discriminate between minor body rotations at low yaw velocities that account for a smooth flight path, and fast body turns at higher yaw velocities that cause a 'jerky' flight. Therefore, I need to define what events of the yaw velocity profile can be classified as turns. Discrete events are defined as turns by applying several increasing thresholds. While pursuing the target that moved on a smooth circular path in my experimental setup, chasing blowflies exhibit smooth and continuous body rotations which are overlaid by brief and rapid body turns of varying amplitudes. The average yaw velocity during the inter-turn intervals is around 940°/s (Fig. 3.9C). The mean peak turning (i.e. yaw) velocities that are reached during the turns, sitting on top of the large, continuous inter-turn yaw rotations, are considerably smaller than those reached during cruising flights (Fig. 3.5A). During cruising flights the flies perform rapid, stereotyped body turns with high angular velocities, starting from close to 0°/s in the inter-turn intervals (cf. Fig. 3.4.C). These turns are called saccades, by analogy to fast 'saccadic' human eye movements (Collett and Land, 1975). During these body saccades the flies change their body orientation by up to 90° (see also Schilstra and van Hateren, 1999; Tammero and Dickinson, 2002a). Hence, the two flight modes – chases and cruising flights – differ considerably with respect of their flight dynamics: whereas the flight trajectories exhibited during chasing are smooth in nature, cruising flights are

characterised by sequences of sharp saccades.

In principle, smooth pursuit is possible when new information about the error angle of the target on the retina is supplied continuously and transformed into body rotations. Hence, smooth movements are used to keep the target fixated in the frontal part of the retina. At the beginning of a chase, an initial rapid turn serves in many cases to bring the target into the frontal part of the visual field (Fig. 3.4A). Depending on the starting orientation of the fly relative to the target, this initial turn may reach high angular velocities as are typical for saccades. My results are in full accordance with a previous modelling study that is based on behavioural experiments which revealed that in male blowflies both types of pursuit response (smooth and saccadic) can be generated by a single smooth control system (Boeddeker and Egelhaaf, 2003; Boeddeker and Egelhaaf, 2005). In this modelling study, a large error angle may, for instance, occur under circumstances where the target is displaced rapidly on the pursuing fly's retina, e.g. at the beginning of a chase. This large error angle is converted into a large turning response, a so-called catch-up saccade, to center the target on the frontal eye region. The error angle may yet increase as a consequence of time constants inherent to the control system, or due to muscular dynamics and inertia wich may further increase the turning response (Boeddeker and Egelhaaf, 2005). Thus, a smooth chasing system can generate rapid, saccade-like turns without employing an extra saccade generating mechanism.

My results on chasing behaviour of blowflies are in accordance with previous studies on chasing behaviour of other fly species. A smooth chasing system including the possibility of body saccades has been shown for the small housefly *Fannia canicularis* (Land and Collett, 1974), for the hoverfly *Syritta pipiens* (Collett and Land, 1975) and for the dolichopodid fly *Poecilobothrus nobilitatus* (Land, 1993b). In contrast to these studies, a saccadic chasing system was concluded to account for chasing in the housefly *Musca domestica* (Wagner, 1986b). If a saccadic controller would steer chasing behaviour in *Lucilia*, the velocity profile during the chase should be markedly different: the velocity profile should be quite jerky rather than smooth in nature and the transients in the velocity profile should be intermitted by gaps (i.e. intersaccadic intervals). The yaw velocity during these intersaccadic intervals should fluctuate around 0°/s. However, the analysis of chasing flight trajectories revealed that the average yaw velocity during these intervals is around 940°/s. Although I cannot exclude that the chasing control system is more complex, I conclude on the basis of my behavioural data that the control system guiding chasing behaviour in blowflies is smooth in nature.

Ocular pursuit of small targets in primates

In insects as well as in primates visual pursuit of small objects is characterised by smooth changes in gaze direction (i.e. continuous movements) that may occasionally be interrupted by saccades (i.e. rapid movements). In primates, including humans, a

smoothly moving small target normally evokes a combination of smooth and saccadic eye movements (review: Orban de Xivry and Lefèvre, 2007) depending on the target speed and on the target displacement with respect to the fovea, similar as in chasing blowflies. The image motion across the retina is sensed by the visual system and is eventually transformed into motor signals that move the eye in an effort to match eye and target motion (e.g. Meyer et al., 1985). At low target speeds (<50°/s), the target is kept centred in the fovea by slow eye movements that follow the target smoothly. When the target is displaced outside the visual field, or when target motion is too rapid at higher target speeds, smooth pursuit is interrupted by saccades to centre the target again (Land, 1992; Rashbass, 1961; reviews: Land, 1999; Zeil et al., 2008). The smooth pursuit system of primates uses mainly target velocity as an important input variable, but also has target position as input. The saccadic system uses predominantly target position as input, but also employs target velocity (Land, 1992; Rashbass, 1961). The smooth and the saccadic components of pursuit eye movements in primates are traditionally thought to be controlled by distinct neural systems. However, recent findings reveal a functional and anatomic linkage between the two systems and suggest that the pursuit system has a functional architecture very similar to that of the saccadic system (de Brouwer et al., 2001, de Brouwer et al., 2002a; Gardner and Lisberger, 2002; Krauzlis and Stone, 1999). Recent studies suggest that it may be more accurate to consider the smooth pursuit eye movements and the saccadic eye movements as different outcomes from a shared cascade of sensory–motor functions (review: Krauzlis, 2004).

Optomotor responses

Whenever a fly - or another animal - moves in the environment, there is continuous image flow over the retina. This so-called optic flow is evaluated by the visual systems of many animals. Components of the optic flow are assumed to form an input to the optomotor control system. When freely flying blowflies are confronted with a large-field rotating environment such as a grating that moves around the flight arena, the flies compensate to some extent the rotation of the background (corresponding to an apparent unintended self-rotation) by turning responses in the direction of the visual motion stimulus (Fig. 3.4B). This compensatory flight behaviour is called optomotor following response (i.e. optomotor flight). The optomotor response is a well-studied behaviour in insects (Götz, 1975). By mediating correcting motor responses, the optomotor turning response is thought to stabilise a straight path of movement by compensating rotations caused either by external disturbances or internal asymmetries in the motor system (reviews: Collett et al., 1993; Hengstenberg, 1993; Kern and Egelhaaf, 2000; Strauss and Heisenberg, 1990; Wehner, 1981). During optomotor following the fly's yaw velocity and forward velocity is increased with increasing background velocity (Fig. 3.5). This increase depends on background velocity. A similar result was obtained in the hoverfly *Syritta* where the yaw velocity increased roughly linearly with the slip speed of the pattern across the fly's retina (Collett, 1980a). In addition, the optomotor stimulus

influenced the translational velocity of *Syritta* (Collett, 1980b).

In primates (including humans), coherent wide-field motion as may be induced on the eyes when the animal rotates around its vertical axis constitutes the input to the so-called optokinetic system which evokes following movements of the eyes. The OKN-reflex of primates serves a similar function as the optomotor response of insects: By counterdirected eye movements the OKN compensates image slip induced by large-field motion, such as occurs during self-motion. Together with the vestibulo-ocular reflex (VOR), which provides fast compensation for head rotation, the OKN helps to stabilize gaze when the head and the body move, such as during self-motion. The OKN-reflex is characterised, like the pursuit of small targets, by smooth eye movements intermitted by relocating saccades (Ilg, 1997).

Cruising behaviour

The flight trajectories of cruising flights, i.e. during spontaneous flights in a stationary environment, are characterised by rapid saccadic turns in both directions with high angular velocities, which are intermitted with periods of little or no rotation (Fig. 3.4C). Many insects, such as different fly species, employ this saccadic viewing strategy (e.g. Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999; Wagner, 1986a; Wagner, 1986b; Wagner, 1986c; review: Land and Collett, 1997). During cruising in the circular flight arena used in my study, male *Lucilia* had average forward velocities of approximately 0.4m/s. This is similar to the mean horizontal flight velocities of about 0.5m/s of *Calliphora* reached inside a rectangular flight arena of similar size (Schilstra and van Hateren, 1999). Much larger translation velocities are reached in larger flight arenas, such as in flight tunnels (Kern, pers. communication).

Flies use the saccadic turns of their body and head to shift the gaze during flight. Thus the gaze is kept basically fixed within the straight-flight intervals between saccades (Land, 1973; Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999). The translatory optic flow generated on the eyes during these intervals can be used by the nervous system to extract information about the spatial layout of the environment (Karmeier et al. 2006; Kern et al., 2005; Kern et al., 2006). In female *Calliphora* the intersaccadic intervals during cruising flights may be as small as 50ms (Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999). I found that in male *Lucilia* the mean duration of these inter-saccade-intervals is as small as 30ms (Fig. 3.9A). Flies turn their head within saccades on average approximately 30% faster than their body. Consequently, head saccades are shorter (van Hateren and Schilstra, 1999) and the head intersaccadic intervals - the periods of stable gaze - are prolonged compared to the periods where the body is kept stable. The retinal image flow evoked by translation, containing information about object distances, is confined to low frequencies. This flow component can be derived from the total optic flow between saccades because the residual intersaccadic head rotations are small and have relatively high frequencies (Kern et al., 2005; Kern et al., 2006). That

behaviourally relevant optic flow information can be decoded within the short intersaccadic intervals is suggested by a combined electrophysiological and modelling study on a population of motion-sensitive visual fly interneurons. It could be shown that an integration time of only 5ms is sufficient to decode accurately the animals' rotation axis (Karmeier et al., 2005).

Similar as the saccadic body turns in flies, primates shift their gaze actively in a sequence of saccades towards interesting locations in a scene. Between the saccades, the detailed analysis of visual information requires the fixation of images on the fovea (Land et al., 1999; Loftus, 1972; Schlingensiepen et al., 1986). During saccades, the perception of motion is assumed to be suppressed (Bridgeman et al., 1975; Burr et al., 1982). This saccadic scanning is in primates the main way in which visual information is selected to solve visual tasks (review: Liversedge and Findlay, 2000).

Characteristics of yaw velocity transients during optomotor and cruising flights

Comparing the yaw velocity profiles of optomotor flights and cruising flights shows that optomotor flights generally display much smaller fluctuations in yaw velocity (Figs. 3.4, 3.5A). Since optomotor flights differ in this aspect so much from the saccadic cruising flights, the optomotor responses may be guided by a smooth control system. The average yaw velocity during cruising flights is around 0°/s, and the blowflies reach peak saccadic yaw velocities of up to 3000°/s. In optomotor flights, the flight trajectory of the fly fluctuates around an offset velocity close to background velocity. In addition, the mean peak turning velocities that are reached during the optomotor flight are substantially smaller than those of body saccades performed during cruising flights (Fig. 3.5A).

From this comparison the question arises, whether the rapid saccadic turns during cruising flights and the smaller turns exhibited during optomotor flight are elicited by different mechanisms. So far, this question cannot be answered, although much is known about the control of saccades during flight in flies (see below). I hypothesize that, since the dynamics of both types of turning behaviour differ much, these are likely to be elicited by different mechanisms. Possibly, the somewhat faster yaw turns that are exhibited during optomotor flight (Fig. 3.4B) may consequence from oscillations of the feedback control system that, dependent on its parameters, can get unstable under certain circumstances. For instance, in a simple feedback pursuit model delays can lead to instability if the gain is high, which can be seen by oscillations (Land, 1992). Thus, the larger body turns during optomotor flight may be expressions of the control system operating at the brink of instability (see also Warzecha and Egelhaaf, 1996). Similarly, the phenomenological model of the chasing controller exhibits under certain circumstances oscillations (Boeddeker and Egelhaaf, 2003).

The saccades as exhibited during cruising flight have been characterised in several studies in tethered or free flying flies: The dynamics of saccades in *Drosophila* are

proposed to be tuned by the amount of rotational feedback provided by the halteres (Dickinson, 1999; Sherman and Dickinson, 2003). The halteres are modified hind wings that act as a mechanical sense specialised to detect body rotations mainly in the high frequency range (Nalbach and Hengstenberg, 1994). Since saccade dynamics are fairly stereotyped, even in total darkness, vision is supposed to play a minimal role in terminating saccades (Bender and Dickinson, 2006b). Similarly, in *Musca*, the time course of the saccade-like turns does not differ significantly in totally blinded and unimpaired flies (Wagner, 1986c). In *Drosophila*, there are indications that at lower angular velocities to which the haltere system is less sensitive, the visual system does provide feedback to flight stability (Sherman and Dickinson, 2003). It is still unknown what neural control systems elicit the saccades during cruising flights. However, it has been concluded for *Drosophila* that saccadic turns can be triggered visually by image expansion in the lateral visual field (Bender and Dickinson, 2006a; Tammero and Dickinson, 2002a; Tammero and Dickinson, 2002b).

Interaction of the optomotor system and the chasing system

While a male blowfly chases another fly in a textured environment, two control systems may be active: First, the chasing controller guiding the chase by generating appropriate turning commands. During self-motion, such as during chasing, the occurring large-field image displacements may activate, second, the optomotor controller, which may try to stabilize the flight path by generating turning commands as well – though in the opposite direction. Thus the two control systems may be in conflict with each other, and this might impair the chasing performance.

How do chasing male blowflies deal with the potential simultaneous activation and then inevitably competing functioning of optomotor and chasing controllers? This question was already formulated in the 1950s by von Holst and Mittelstaedt (1950) in the following way: how can an animal with an optomotor system make intentional turns without automatically correcting (and thus negating) them?

To address the issue of how male blowflies cope with optomotor stimulation during chasing manoeuvres I analysed on the one hand behavioural experiments and on the other hand the detailed flight trajectories obtained under different optomotor stimulation conditions. I manipulated the environment of chasing flies such that the optomotor stimulation was decreased or increased with respect to the normal stationary environment. This was done by moving a large-field grating around the flight arena. The employed five different background velocities are a stationary background as well as slow and fast background motion that moved in the same or in the opposite direction as the target, respectively.

In the *behavioural experiments* I investigated the number of captures and the flight frequency of male blowflies during simultaneous optomotor stimulation. I found that large background velocities reduced the number of captures to about one third.

However, when counting the number of flies that were actually flying, I found a significant decrease (about one half) of the flight frequency during fast background motion (Fig. 3.6). In summary, these results suggest that the catching success does not deteriorate as a consequence of background motion after the flies initiate a chase.

Analysis of the flight trajectories

Does the optomotor system have an impact on the fine structure of the time course of chasing flights? Optomotor stimulation alone strongly affects the fly's yaw- and forward velocity (see above; Collett, 1980a). Therefore, one might expect that a chasing fly could be somewhat retarded by an experimentally decreased, and accelerated by an experimentally increased optomotor stimulation. However, no such effect could be found. In addition, no consistent effect of different background motion velocities can be found on the fly's mean yaw and forward velocity as well as on the turning frequency when analysing successful and non-successful chases (i.e. C-chases and P-chases; Fig. 3.7).

Another parameter was analysed to quantify the chasing performance: the error angle. A small error angle indicates that the pursuer keeps the target fixated frontally. In chasing males, the retinal image of the target was found to reside within the frontal region of the eye (Fig. 3.8 B,C; see also Boeddeker et al 2003; Collett, 1980a; Land, 1993a; Land, 1993b; Land and Collett, 1974; Wagner, 1986b; Wehrhahn, 1979; Zeil, 1983). This frontal region, the so-called acute zone, has specialized anatomical and physiological properties in male flies which are assumed to be advantageous to chasing behaviour (see chapter 2.2.1; Burton and Laughlin, 2003; Francescini et al., 1981; Gilbert and Strausfeld, 1991; Gronenberg and Strausfeld, 1991; Hardie et al., 1981; Hornstein et al., 2000; Land, 1997; Land and Eckert, 1985; Strausfeld, 1991; Trischler et al., 2007). If chasing behaviour were affected by the experimentally modified optomotor stimulation, the fixation performance most probably would deteriorate and the error angle might eventually enlarge. My results reveal that under all background motion conditions the mean error angle lies well within the frontal visual field.

Altogether, my results indicate that large-field motion has no consistent impact on the fine structure of chasing behaviour. The success (i.e. capture) or failure (no capture) of chases might not be influenced by an interaction of the optomotor system but might depend on other factors such as the size or speed of the target as was shown by Boeddeker et al. (Boeddeker et al., 2003). In addition, the fly's initial starting position and the orientations of the fly relative to the target and, possibly, the male fly's actual state of fitness may affect chasing performance.

Interaction of the optomotor system with systems controlling other behaviours in insects and primates

The problem of a potential interference of the optomotor system with other behavioural systems is not restricted to flies. This holds true for other species of flies as well as for insects and vertebrates. Although this interference and its consequences has been the subject of many investigations, I will mention here only few examples. For instance, the impact of the optomotor system was investigated for another visually guided control system in flies, the object detection and fixation system.

The object detection and fixation system

Whereas the chasing system guides chasing behaviour exclusively in male flies, another control system underlying 'object' detection and fixation is assumed to exist in flies of both sexes. This control system is thought to be concerned with detecting small-field visual cues such as leafs to avoid collisions or to land on them. Thus, when a fly passes a nearby object, it can use motion cues, i.e. the relative motion information at the edges of objects, to detect the objects in front of a structured background (in bees: Kern et al., 1997; in flies: Kimmerle et al., 1997). In previous studies tethered flying flies (*Lucilia, Musca*) have been shown to turn towards an object (Kimmerle et al., 1997) and to fixate this object in the frontal part of the visual field (Reichardt et al., 1983; Virsik and Reichardt, 1976). Similar behavioural fixating responses to small objects have been recently found in *Drosophila* (Duistermars et al., 2007). Based on behavioural experiments, a phenomenological orientation theory was established to describe object fixation. In particular, it was shown that a tethered fly, which yaw turns were measured by transformation into voltage, visually fixates an object and the fly's fixation error angle is proportional to the object's angular velocity (Virsik and Reichardt, 1976). Similarly, in chasing male blowflies, the target's error angle controls the fly's yaw rotation (Boeddeker and Egelhaaf, 2005).

Despite this similarity, the function and underlying neural mechanisms of chasing and object fixation behaviour are different. The neuronal substrate mediating chasing behaviour in male blowflies is most likely constituted on the visual input site by the so-called male-specific visual interneurons (MLGs) that have been demonstrated to be small-field sensitive and direction selective (e.g. Gilbert and Strausfeld, 1991; Hausen and Strausfeld, 1980; Trischler et al., 2007; Wachenfeld, 1994). Furthermore, the receptive field of MLGs covers the frontal region of the dorsal visual field. By contrast, the FD-cells (Figure-Detection-cells), which are assumed to form a part of the neuronal circuit underlying object fixation presumably exist in flies of both sexes. The receptive fields of FD-cells cover within the ventral visual field frontal or lateral eye regions. Similarly as MLGS, FD-cells are direction selective and small-field sensitive; however, the MLGs seem to be tuned to smaller targets than FD-cells (Egelhaaf, 1985b; Egelhaaf, 1985c; Wachenfeld, 1994).

In situations where the fly turns towards an object, the whole background drifts in the opposite direction which might activate the optomotor system inducing a counterdirected turning command. As during chasing behaviour, in these situations the fly might face the problem of two control systems (the FD-system and the optomotor system) being in conflict with each other. Several studies – which partially differ in experimental methodology – investigated the interaction of the optomotor system and the object fixation system in several species of flies. In earlier studies it was concluded that the influences of the object and the background are additive (Virsik and Reichardt, 1976). Later studies revealed that both systems have different dynamic properties: Whereas the object detection and fixation system is most sensitive to small-field stimuli moving at high temporal frequencies, the optomotor system is most sensitive to large-field stimuli changing velocity at low frequencies (Duistermars et al., 2007; Egelhaaf, 1987; Egelhaaf et al., 1988; review: Hausen and Egelhaaf, 1989). Therefore, both control systems may operate in a given behavioural context without impairing one another. A more detailed discussion about the dynamic separation as mechanism of interaction of the two control systems follows below.

Examples of interactions of the optomotor system with other behavioural systems

The influence of visual and acoustic stimuli on optomotor course control has been studied on walking crickets (*Gryllus bimaculatus*) (Böhm et al., 1991). It was found that the large-field visual cues (i.e. optomotor stimulus) additively shifted the direction of walking elicited by the calling song: The turning towards the phonotactic stimulus was enhanced in front of a stationary grating, was further enhanced in front of a grating moving in the same direction as the phonotactic stimulus and was reduced in front of a grating moving in the opposite direction indicating that the optomotor stimuli have an impact on the phonotactic orientation in an additive way (Böhm et al., 1991).

The visually guided head and body movements of mantids (*Tenodera australasiae*) have been studied using various small targets and large-field backgrounds as visual cues (Rossel, 1980). Praying mantids exhibit smooth as well as saccadic pursuit strategies in pursuing tasks. The extent to which either pursuit strategy is actually employed depends mainly on the features of the background and to some extent also on target velocity. For instance, targets that move at low velocities in front of a homogenous background are tracked by smooth head movements, whereas a stationary, textured background causes the system to switch from smooth to saccadic tracking. Rossel (1980) concludes that the small-field target pursuit responses and the large-field optomotor following response are not combined additively; Rather the pursuit response to small targets is weighted more strongly, but competing background motion can strongly affect smooth target pursuit. However, this limitation of the smooth pursuit system is compensated by switching to a strategy of saccadic tracking (review: Kral, 2003).

A recent study investigated the interaction of optomotor stimuli and olfactory cues in *Drosophila*. Flies tracked motion signals more closely in an odour plume, and the attractive odorant increased the ability of flies to stabilize image motion. This indicates that olfactory signals can improve the salience of visual stimuli and can enhance the optomotor gain. This enables an animal to fly straight up a plume and approach odiferous objects (Chow and Frye, 2008).

Interaction of the optomotor system and target pursuit system in primates

While primates perform visual pursuit of a small object, they may encounter a similar problem of two potentially conflicting control systems as chasing flies: the eye movements pursuing a small target that moves in front of a structured background may be counteracted by following eye movements induced by the resulting wide-field motion in the opposite direction. This issue of the interaction between OKN and target pursuit has been addressed in a large number of studies. Numerous results indicate that target pursuit is clearly influenced by simultaneous OKN-stimulation. However, the results are inconsistent with respect to details, which might result from methodological differences between studies.

Some behavioural studies indicate that target pursuit eye movements in monkeys and humans are affected by a stationary or a moving visual background (Collewijn and Tamminga, 1984; Keller and Khan, 1986; Kimming et al., 1992; Masson et al., 1995; Mohrmann and Thier, 1995; Niemann and Hoffman, 1997). Masson and colleagues (Masson et al., 1995) found that a background moving into the same direction as the target increased pursuit velocity, whereas background moving in the opposite direction decreased the pursuit eye velocity. Furthermore, a brief background perturbation during targt pursuit evoked a transient increase of eye velocity into the direction of the perturbation (Lindner et al, 2001; Schwarz and Ilg, 1999). Born and others showed that the direction of pursuit eye movements was shifted in a direction opposite to the background motion (Born et al., 2000). By contrast, in another study a drifting background was found to enhance target pursuit performance, irrespective of its direction of motion (Spering and Gegenfurtner, 2007). Several studies found inhibitory effects on target pursuit velocity by a textured background that is stationary (Mohrmann and Thier, 1995; Spering and Gegenfurtner, 2007) or moving opposite of the target (Masson et al., 1995). Altogether, there are many indications that in primates visual target pursuit is significantly influenced by simultaneous wide-field stimulation. Hence, at least at the behavioural level, the two control systems apparently seem to affect each other.

To summarize the many investigations of control system interactions, many studies on a wide range of species demonstrated a considerable impact of the optomotor system on the pursuit of small targets. In the studies employing mantids, monkeys and humans, the subjects were stationary during the experiments and visually pursued a moving object against a stationary or moving background. This pursuit task demanded the detection of the target against the background, and involved

'only' the rotation of eyes and head. In my study, the pursuit task of male blowflies is much more demanding, since chasing behaviour not only requires target detection and pursuit but additionally includes the self-motion towards the target in front of a textured background in very fast flight manoeuvres. Nonetheless, in contrast to the many other systems, I found no consistent impact of optomotor stimulation on the performance of chasing behaviour in male blowflies, which emphasizes the chasing system to be a very efficient and reliable chasing controller.

Characteristics and integration of the flight control systems in blowflies

I will jointly discuss the characteristics of the chasing, optomotor and cruising behaviour, their mutual impact, as well as the results of other studies. Fig. 3.11 shows a diagram that denotes the functional characteristics of the different control systems mediating different types of turning behaviour and their possible interactions.

Saccade generator

Cruising flights have been described in males and females of several fly species (*Calliphora, Drosophila, Lucilia, Musca*) (Fig. 3.4C; Bender and Dickinson, 2006b; Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999; Wagner, 1986c). It is assumed that a kind of saccade generator may be active during cruising flights which produces fast turns with stereotypic time courses but variable amplitudes (Fig. 3.11). Saccade dynamics have been described in blowflies and in fruitflies. During saccades, flies can reach maximum angular velocity values of approximately 2000°/s and change their body orientation by up to 90° in about 50-100 ms (Bender and Dickinson, 2006a; Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999). A recent study modelled the visual course system of the fly. The flight performance of this so-called 'Cyberfly' was tested in a flight arena employing either a saccade controller or an optomotor controller (Lindemann et al., 2008). During cruising flights the Cyberfly successfully avoids collisions with obstacles when using a saccade generator and fails to avoid obstacles when employing an optomotor controller. However, the performance of the Cyberfly strongly depends on the textural properties of the environment (Lindemann et al., 2008). The visual course system of the fly is assumed to receive sensory input from identified motion sensitive visual interneurons (Hausen, 1982a; Hausen, 1982b). Their model counterparts providing this input into the saccade generator of the Cyberfly are calibrated such that they are capable of extracting information from the translation induced optic flow in the intersaccadic intervals. Hence, this neuronal input model allows the Cyberfly exploiting the saccadic gaze strategy just like real blowflies (Lindemann et al., 2005).

Optomotor system

The optomotor system is assumed to exist in male and female flies of different fly

species (Collett, 1980a; Srinivasan and Bernard, 1977). During the rather smooth optomotor responses, the flies do not execute high-amplitude saccades (Fig. 3.4B). This indicates that the optomotor system inhibits the generation of saccades (Fig. 3.11). The optomotor system may integrate the optic flow over a time window larger than the duration of short saccades. This large time window may correspond to, for instance, the duration of internal or external disturbances on the animal's flight path (Warzecha and Egelhaaf, 1996). This conclusion is corroborated by behavioural experiments in freely flying and walking flies that were monocularly blinded. The monocular flies tended to turn slightly towards the side of the open eye which was concluded to be mediated by the optomotor system (Kern and Egelhaaf, 2000).

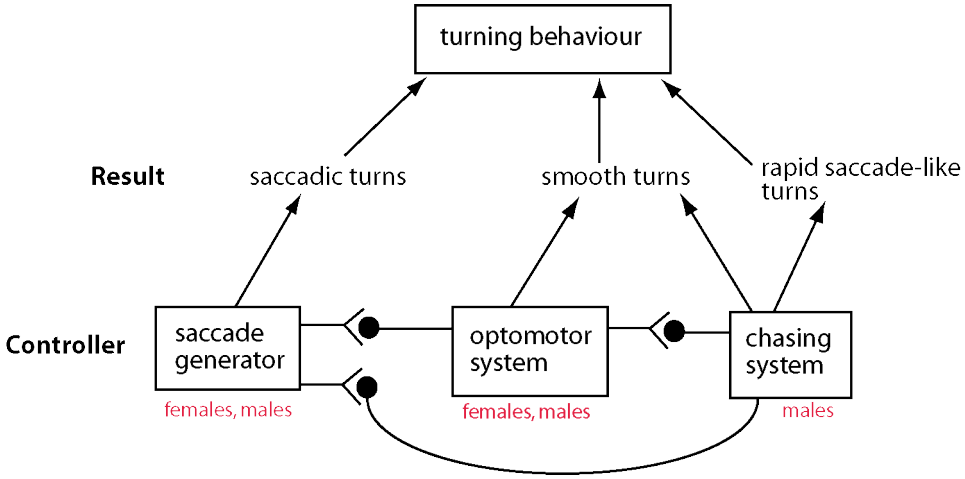


Figure 3.11 Diagram of the possible interactions between the chasing system, the optomotor system and the saccade generator. Stereotyped high-amplitude saccadic turns are generated by male and female flies while the fly is cruising, i.e. flying around without any obvious goal. The optomotor system that is presumed to exist in males and females generates smooth turns and reduces the generation of saccades. A signal of the male-specific chasing system has inhibitory effects on the optomotor system and reduces the generation of saccades. During chasing flights, the chasing system generates smooth turns and, occasionally, to compensate for large retinal fixation errors of the target, it generates rapid turns, i.e. the catch-up saccades.

Chasing system

Since the optomotor stimulation has no significant impact on the chasing performance (Fig. 3.7), the male-specific chasing system might have inhibitory effects on the optomotor system (Fig. 3.11). In addition, since chasing behaviour of blowflies is smooth in nature, and sharp saccadic turns occur only rarely (Fig. 3.4A), the chasing system might as well inhibit the generation of saccades. As previously mentioned, the saccade-like turns that occur during chasing are thought to be an emergent property of the smooth chasing system (Boeddeker and Egelhaaf, 2003; Boeddeker and Egelhaaf, 2005). The question may arise, whether the smooth chasing trajectories in the present study might be the consequence of the specific

experimental setup, since the chased target moved on a circular track. In natural situations, potential targets usually do not constantly move on tracks as the artificial target in my experiments. These fly-fly chases are characterised by erratic flight manoeuvres with sudden changes in flight direction (cf. Fig. 2.1; see also Collett and Land, 1975; Land and Collett, 1974, Wagner 1986b). However, the virtual blowfly that implemented a smooth chasing controller was shown to be capable in pursuing targets that move like a real fly (Boeddeker and Egelhaaf, 2003; Boeddeker and Egelhaaf, 2005). During these erratic chases, as they may occur when chasing real flies, target displacements towards lateral positions on the retina are compensated by catch-up saccades. The flight trajectories of the virtual blowfly are smoothed when the model is supplied by an additional input, the target's velocity (Boeddeker and Egelhaaf, 2005).

The *neuronal substrate* underlying the visual small field selective systems have been analysed in several flying insect species. Newly described visual interneurons in hoverflies have sophisticated receptive field properties reminiscent of neurons in the mammalian visual cortex. These small target motion detectors (STMDs) respond selectively to the motion of small objects. Some of these STMDs respond to target motion even during a large range of ongoing background motion stimuli; others are inhibited by the motion of a background pattern. These cells are well-suited to compute motion of conspecific females that are chased by males (Barnett et al., 2007; Nordström et al., 2006). Small target selective neurons were also described in dragonflies (Frye and Olberg, 1995; O'Carroll, 1993). These neurons are strongly directionally selective. It is suggested that the behavioural function of these specialized target detectors is to steer the dragonfly during prey-tracking so as to fix the position of the prey image on the retina. As mentioned previosly, recent studies on *Calliphora* and *Sarcophaga* characterised the MLGs (Male Lobula Giant neurons) most likely as the neuronal substrate mediating chasing behaviour. These large male-specific visual interneurons are loalized in the lobula, the third visual neuropil of the fly's brain (cf. Fig. 2.10; Gilbert and Strausfeld 1991; Hausen and Strausfeld 1980; Strausfeld 1991; Wachenfeld 1994). On the basis of naturalistic stimuli it was found that the MLG1 neuron, one prominent neuron of this ensemble of male-specific cells, shows a distinct direction selectivity and complex nonlinear response characteristics to the joint occurrence of multiple visual parameters of the target including size, position and velocity and their variation over time (see chapter 4; Trischler et al., 2007).

Some studies engaged in understanding the *computations that underlie small target selectivity*, as is necessary during target pursuit, for instance during chasing and during object fixation behaviour. Computational models for target discrimination can rely on lateral inhibitory interactions around a central element in locust neurons (Rowell et al., 1977). In a new model of the object detection in the fly visual system the analysis of the neuronal computations underlying the detection of small objects is based on electrophysiological experiments on Figure Detection cells (FD-cells)

(Hennig et al., 2008). The authors conclude that distributed dendritic and presynaptic inhibition of retinotopic input elements is the most plausible wiring scheme for the neuronal ensemble of FD-cells. Another computational model of fly visual interneurons that detect small moving targets and reject background motion is based on lateral inhibition connections and fast temporal adaptation (Wiedermann et al., 2008).

Mechanisms used for the integration of control systems

A range of studies in different species have led to rather divergent conclusions about how two, potentially conflicting, behavioural responses should be combined. In general, it seems that neuronal circuits that provide a solution to this problem are necessary for the proper function of nearly all sensory systems, and they exist at various levels in sensorimotor systems (Crapse and Sommer, 2008). For instance, not only chasing male flies, but most likely all moving animals are concerned with the task to distinguish sensory input generated by active self-movement (for instance a turn towards a target) from sensory input generated by external sources (for instance a passive rotation by a gust of wind). It seems plausible that different animals might solve this task in different ways, dependent on the behavioural context. I will discuss now several schemes that were proposed as potential integration mechanisms of control systems, and I try to conclude, in addition to above implications, from my behavioural results on mechanisms underlying the integration of chasing and optomotor control in male blowflies. The question of the relation between the chasing behaviour and the optomotor following response was examined in an earlier study in male hoverflies *(Syritta)* (Collett, 1980a). With regard to the interaction of the two control systems several models - originally developed in the 1950s (Mittelstaedt, 1951) - were assessed: the 'additive', the 'efference copy' and the 'follow-on' scheme.

Follow-on scheme

In the 'follow-on' scheme, the command of the chasing controller to turn towards the target is actually controlled via the optomotor response. Thereby, this turning command is injected at an appropriate point into the optomotor pathway in order to change the set point of the optomotor system (Fig. 3.12A). As a consequence, the latter executes the desired turn towards the target. A disadvantage of this follow-on scheme is that the chasing is subject to the same delays and time constants as the optomotor system. Experimental and modelling studies on the two control systems revealed considerable differences in the time constants of their intrinsic low-pass filters: Whereas the time constant of the optomotor system was approximated to be in the range of 750ms (Warzecha and Egelhaaf, 1996), the time constant of the yaw speed control pathway of the model of chasing behaviour was as small as 15ms (Boeddeker and Egelhaaf, 2005). When considering these assumptions, the follow-on scheme can not account for the data of my study.

Efference copy and corollary discharge

The second model is the 'efference copy' model (von Holst and Mittelstaedt, 1950), which is similar to the 'corollary discharge' scheme (Sperry, 1950) (Fig. 3.12B). According to the efference copy scheme, a copy of every turning command of the chasing system is generated during chasing. This 'efference copy' is sent to the optomotor system in order to cancel out the neuronal responses to the visual consequences of the turning command of the chasing system. In principle, the nervous system of an animal may be able to predict the visual consequences of self-motion in a stationary environment, but it is not likely that the nervous system can predict the visual consequences of self-motion in a moving environment, because the animal has no *a priori* knowledge of external global image displacements. If the efference copy scheme would apply to the chasing of male blowflies, the error angle between the target and the chasing fly should be affected consistently by a decrease or increase of optomotor stimulation with respect to stationary conditions. Since this is not the case, this scheme can not explain the interaction between chasing and optomotor controllers.

Whereas the efference copy is provided by a signal that effects at a relatively 'early' stage of the targeted sensory pathway, the corollary discharge signals can target different stages of the sensory pathway (Crapse and Sommer, 2008). Corollary discharge schemes are thought to exist at two functional levels, a lower- and a higher-order level (Crapse and Sommer, 2008). Low-order-level circuits have mainly been described so far in invertebrates and prevent maladaptive responses by functions such as reflex inhibition and selective filtration of sensory information. One example is the interaction of the optomotor response with the acoustic avoidance behaviour (i.e. turning away from a sound source) in locusts (Robert and Rowell, 1992). The authors found indications that acoustic avoidance turns are temporarily independent of visual information indicating that the optomotor response is reduced by corollary discharge. Further examples are the inhibition of the escape-reflex during feeding of crayfish (Edwards et al., 1999), or the directed filtration of self-generated songs in crickets (Poulet and Hedwig, 2006). These mechanisms are often a type of access control where the 'accurate' time point is highly critical. They generally implement a gain mechanism that modulates a reflex or gates the input of sensory information at the periphery. High-order-level circuits have been described in higher vertebrates. These corollary discharge circuits mediate sophisticated predictive computations in the fields of sensorimotor learning, perceptual stabilisation and coordination tasks. Examples are the visuosaccadic system in monkeys which carries spatial and temporal information about upcoming saccades (Schall, 2004; review: Sommer and Wurtz, 2008) and the auditory system in birds. Male juvenile birds learn singing by listening to other adult birds as tutors. The young bird is thought to fine-tune its song by comparing auditory feedback of its own song with copies of tutors' songs that are stored in memory. The errors between the memory and the actual acoustic feedback are corrected by adjustment

of the bird's own singing motor commands (review: Brainard and Doupe, 2000). These high-order circuits are generally multidimensional and they encode more parameters than just time (Crapse and Sommer, 2008).

The concepts of 'efference copy' or 'corollary discharge' resemble models that were designed in recent modelling studies to explain the integration of phonotaxis and optomotor responses in crickets. These modelling studies are based on behavioural experiments in crickets, where the turning tendency towards optomotor and phonotactic stimuli was primarily concluded to combine additively (Böhm et al., 1991). Modelling the cricket's behaviour revealed that some inhibitory interactions are most plausible to explain how crickets integrate phonotaxis and optomotor stimuli (Webb and Reeve, 2003). The inhibition is represented in the model by a biologically realistic 'shunting' mechanism, which counteracts the optomotor response up to the amount and in the direction expected during a phonotactic turn. However, more recent studies using robots to model the cricket's behaviour reveal that another model, the 'forward model', which is closely related to the efference copy scheme, is a more plausible mechanism to explain the interaction of the two control systems (Webb, 2004). The essential idea of forward models is that nervous systems are capable to predict the sensory consequences of movements. This is often associated with higher cognitive capabilities; yet many of the purposes that forward models are thought to serve have analogues in insect behaviour (Webb, 2004).

Additive model

In an 'additive scheme' two control systems are combined additively (Fig. 3.12C). Collett (Collett, 1980a) proposed that this scheme explains the interaction of chasing and optomotor response in male hoverflies (*Syritta*). According to this model, both control systems are independent until their commands come together at a final pathway. However, to give the required chasing performance, the gain of the chasing system must be larger than that of the optomotor system (Collett, 1980a). Collett found in *Syritta* that the gain of the chasing system is different from that of the optomotor system, in that it remains constant within a broad range of tested frequencies. By contrast, the optomotor system was found to respond best to low frequencies of up to approximately 0.5 Hz, and its gain decreases between 0.5-5 Hz (Collett, 1980a). Subsequent studies in *Musca* revealed similar dynamic differences on the object-fixation system and the optomotor system (Egelhaaf, 1987; Egelhaaf et al., 1988). Whereas the small-field sensitive object-detection and -fixation system shows its strongest responses to transient object movements at high frequencies (up to 4Hz), the large-field sensitive optomotor system responds best to frequencies around 1-2Hz due to low-pass filtering in the visual motion pathway (Egelhaaf, 1987; Egelhaaf et al., 1988; Hausen, 1982a; Hausen, 1982b; review: Hausen and Egelhaaf, 1989). Recently, similar dynamic properties of these two control systems were found in the fruitfly *Drosophila* (Duistermars et al., 2007; Sherman and Dickinson, 2003). These differences in the dynamical properties of the small-field

system (i.e. the object -detection and -fixation system) and the large-field system (i.e. the optomotor system) could be a simple strategy to almost eliminate the unwanted optomotor influence on active turns, since the generation of rapid turns (head or body saccades) during object fixation may induce motion of the environment beyond the dynamic sensitivity range of the optomotor system (Collett, 1980a; Duistermars et al., 2007; Egelhaaf, 1987).

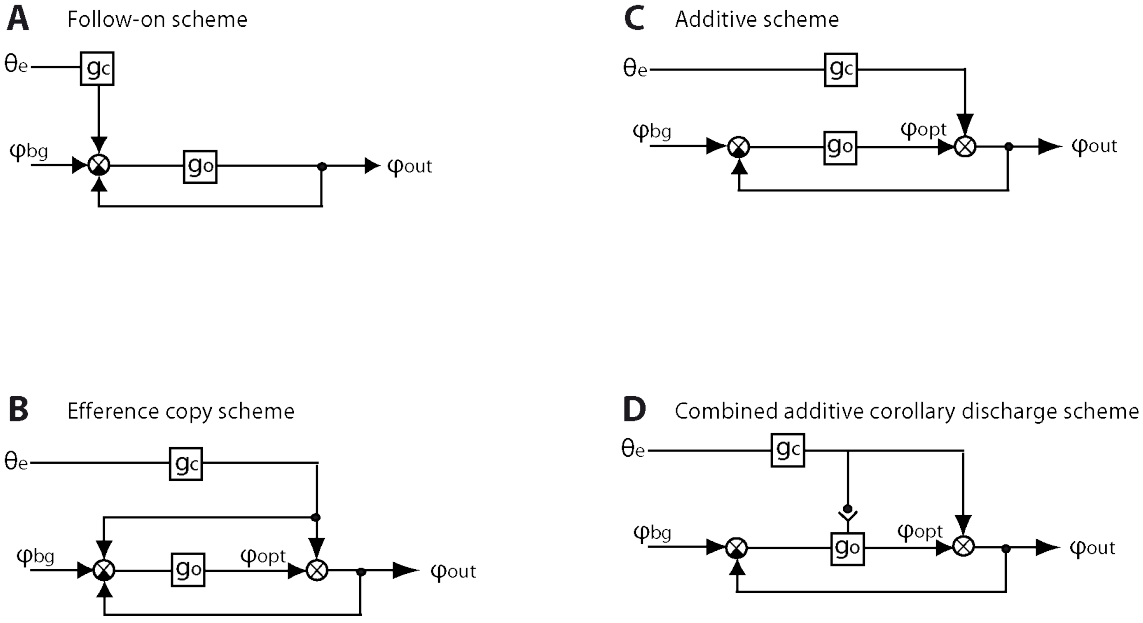


Figure 3.12 Cybernetic models of the possible form of interaction between the chasing and the optomotor system . The input to the chasing system is *θe*, i.e. the error angle of the target relative to the frontal midline of the fly's visual field. *gc* and *go* are the internal gains of the chasing system and the optomotor system, respectively. The input to the optomotor system is provided by *φbg* that is the angular velocity of the environment (i.e. background). *φopt* is the output of the optomotor system, and *φout* is the final output of the two systems, i.e. the final behavioural (turning) response (A,B,C modified from Collett, 1980a).

By using this type of dynamic separation both control systems can, although they combine additively, operate in the appropriate behavioural context quite independently from each other. This functional separation of the two control systems illustrates their adaptation to the relevant behavioural situations: Under free-flight conditions, slow changes of the direction of motion may occur during passive course deviations, e.g. a passive drift as a consequence of a gust of wind, whereas fast changes in flight direction may be caused by active body turns, such as by a reorientation towards an object.

The additive scheme was also proposed to explain in houseflies and fruitflies the integration of the optomotor behaviour with other behavioural components: In *Drosophila*, mechanosensory feedback from the halteres and visual sensory feedback

channels were shown to be combined in the fly's flight control system by a weighted sum, with greater emphasis placed on mechanosensory feedback (Sherman and Dickinson, 2004). Furthermore, visual and olfactory stimuli are shown to modulate wing beat in *Drosophila*, and the responses to bimodal stimuli are nearly identical to the sum of responses to stimuli presented in isolation (Frye and Dickinson, 2004).

Mechanism of the interaction between chasing and optomotor system

Can the additive scheme combined with a dynamic separation of the two control systems explain the present results on simultaneous target chasing and optomotor stimulation in male blowflies? As previously mentioned, the response properties of the optomotor system are found in fly species such as *Musca* and *Drosophila* to be especially sensitive at low frequencies (Egelhaaf, 1987; Duistermars et al., 2007; reviews: Collett et al., 1993; Egelhaaf and Borst, 1993; Egelhaaf and Warzecha, 1999; Reichert, 1993). It is reasonable to assume that the optomotor system in male (and female) *Lucilia* shows similar functional characteristics. On the other hand, chasing is characterised by fast transient movements – it belongs to the fastest visually guided behaviours that can be found in nature. Therefore, the temporal frequency characteristics of the chasing system may be in the high-frequency range. Up to now, the temporal frequency properties of the chasing system have not been analysed yet under open-loop conditions. However, I examined the velocity fluctuations of the input of the chasing system under closed-loop conditions. The velocity fluctuations of the retinal error angle are thought to control the pursuer's angular velocity (Boeddeker and Egelhaaf, 2003; Boeddeker and Egelhaaf, 2005). The analysis of chasing flights demonstrates average retinal target velocities of up to 150°/s (Fig. 3.8E). The power spectrum of this retinal target velocity shows that the chasing system receives frequency input on a broad frequency range: low as well as high frequencies up to approximately 30-50Hz are prominent in the error angle fluctuations during chasing flights (Fig. 3.8F). This indicates that fast, transient target velocities occur during chasing. Due to the constantly moving target as used in my experimental setup, the chasing system receives mainly low-frequency input. In this situation, target fixation performance would deteriorate if the chasing controller would predominantly respond to fast transient movements and not to a constant velocity input. Male blowflies fixate the target well within the frontal eye region even if it moves at a constant velocity. The average fixation error is between 0° and +10°, and considering the standard deviations laying between 7° and 24° (Fig. 3.8C), the target still resides within the frontal eye region assumed to serve chasing behaviour (Land and Eckert, 1985). These results indicate that the chasing control system responds well to constant moving visual stimuli. Hence, the dynamic properties of the chasing system and the optomotor system overlap to a large extent. While the optomotor system may respond to low-frequency input, as it was shown in several previous studies (see above), the chasing system may respond to visual input over a broad frequency range. It is therefore unlikely, that a dynamic separation of the two control systems accounts for a robust chasing performance.

This is underlined considering the artificial experimental conditions employed in my study: While male flies chase the constantly moving target smoothly, the background, as a consequence, also is displaced smoothly in the opposite direction. If no other computational measures were taken by the fly's brain, the optomotor system may respond under these conditions to the large-field motion and, thus, may impede the chasing performance by compensatory yaw body turns.

Which mechanism may then allow male blowflies to execute the chasing behaviour without being impaired by the optomotor system? It is likely that during chasing the turning commands of the optomotor system are subject to some kind of inhibition. This could be realised by reducing the optomotor gain during chasing behaviour. How might this optomotor gain reduction be accomplished? The chasing system receives an 'appropriate' visual input stimulus, which is the error angle of a pursued target. The chasing system then generates the turning command towards the target, which is the system's output signal. A copy of this chasing signal may target the optomotor pathway and reduce its gain (Fig. 3.12D). While the chasing fly turns towards the target, the counterdirected motion of the visual environment delivers large-field motion stimuli to the optomotor system. However, due to its gain reduction, the optomotor system does not generate large optomotor turning responses. As a consequence, the chasing system dominates the overall turning behaviour.

Where in the visual pathway does the copy of the chasing signal interact with the optomotor system? The level at which the copy of the chasing signal targets the optomotor pathway is significant for the chasing performance, because a temporal low-pass filter with a large (about 750ms) time-constant was supposed to be part in the optomotor system (Egelhaaf, 1987; Warzecha and Egelhaaf, 1996). This filtering has been concluded to be accomplished between the lobula plate, the third visual neuropil of the fly's brain, containing the neuronal substrate subserving the input to the optomotor system (cf. Figs. 2.9; 2.13), and the final behavioural optomotor response. If the copy of the chasing signal affects the optomotor gain in terms of efference copy at 'early' levels of the pathway before the low-pass filtering takes place, this signal would be subject to the same large time constant. Then the gain reduction of the optomotor response would be delayed with respect to the chasing signal and thus would partially interfere with chasing performance. For these reasons, it is likely that the copy of the chasing signal reduces the optomotor gain at a later stage in the optomotor pathway in terms of corollary discharge (Fig. 3.12D).

The chasing controller being sensitive to visual motion stimuli of a broad frequency range may denote a good adaptation to natural behavioural situations as they occur when a male fly chases a conspecific: During these chases, the pursued fly may perform fast transient flight manoeuvres (see the initial part of Fig. 2.1). Between these sudden changes of the flight direction there may be as well sequences of movement in a relatively constant direction (see the last part of Fig. 2.1). It cannot be excluded that under outdoor conditions these chasing sequences of relatively

straight flight are performed for even longer duration than in the flight arena used in my experiments with a radius of 0.2m. Therefore, the preference of the chasing system for transient as well as constant motion stimuli may be a good adaptation to the real chasing conditions in natural environments.

The mechanism that controls the gain of the chasing system may depend on several factors. Basically, only the presence of an 'appropriate' visual stimulus (i.e. a small target) constitutes visual input to the chasing system. Moreover, the fly's age could play a role controlling the gain of the chasing system. Several studies denote that physiological and biomechanical maturation influences sexual maturity and chasing performance in male flies: In young male *Sarcophaga*, the onset of mating occurs three days after eclosion and coincides with a rise in the titre of steroids in males and females (Yocum et al., 1987). In young male onion flies, the begin of mating coincides with sperm maturation. Male mating peaks at 6 days of age, which coincides with the plateau production of a male sex peptide (Spencer et al., 1995). Moreover, in very young male flesh flies (*Sarcophaga*), the cuticula may not be hardened sufficiently (Gilbert and Min, 2007) to withstand the large forces on the wings and thorax associated with flight (Sane, 2003). Furthermore, the neuromuscular system of many insects is subject to significant changes following adult eclosion from the pupa (Consoulas et al., 2000). Muscles and their motoneurons involved in eclosion may degenerate and such reorganisational processes may contribute to the lack of successful chasing flights in young male flies.

I have previously discussed in primates two visually driven following behaviours, the smooth pursuit eye movements and the saccadic eye movements. What mechanism explains the interaction of pursuit and saccades in primates? In primates, the exact neural circuit of the control systems mediating pursuit and saccades, respectively, is not yet known. However, recent data led to a proposal (Keller and Missal, 2003; review: Krauzlis, 2004). According to this proposal, the gain of pursuit and saccades is regulated by inhibitory effects on the neurons involved in generating pursuit or saccades, respectively. The sensitivity of the pursuit system to visual inputs depends on a variety of factors and varies in a graded fashion (Krauzlis and Miles, 1996). Thus, unlike saccades, the gating mechanism for pursuit is not all or nothing but graded. This is in contrast to my results in male blowflies, which suggest that during chasing behaviour the generation of saccades is reduced and the gain for the chasing system and the optomotor system differ considerably.

Multisensory integration in insects and vertebrates

Although control systems may in some situations 'be in conflict' for the execution of behaviour, they may in other situations collaborate in order that the animal best achieves its goal. There are many examples in invertebrates and vertebrates for the integration of inputs of several available sensory modalities to control behaviour and to efficiently interact with the environment.

Insects use many sensory systems for the detection and discrimination of events and for directed behavioural responses. There are several examples of interactions of non-visual modalities with the visual system in insects. For instance, in flies, stable flight and course control relies on the synergistic interaction of sensory systems. On the one hand, the optomotor system uses visual information from the compound eyes to stabilize flight; support is constituted by the light-sensitive ocelli that provide input to motion-sensitive neurons that participate in visual course control (Haag et al., 2007; Parsons, et al., 2006). On the other hand, flies possess a mechanical sense specialised to detect rotations, the so-called halteres, which are required for stability reflexes during flight (Dickinson, 1999; Nalbach, 1993; Pringle, 1948; review: Taylor and Krapp, 2008). Similarly, in hawk moths, the antennae that experience Coriolis forces during flight are thought to play a role in stabilizing flight (Sane et al., 2007). To maintain a certain heading direction honeybees and migrating butterflies use, besides the visual input from the eyes, the additional input from the magnetic sense (e.g. Banks and Srygley, 2003; Collett and Baron, 1994; Etheredge et al., 1999; Martin and Lindauer, 1977; Srygley et al., 2006). Further examples of bimodal interactions with the visual system are in fruit flies, where the presentation of specific visual cues enhances the olfactory acuity during searching behaviour (Frye et al., 2003), and in crickets, where the presentation of distinct visual cues enhances the phonotactic acuity of the walking course of females towards males (von Helversen and Wendler, 2000). An important neural substrate for multimodal integration in the insect brain is assumed to be the paired mushroom bodies. The function of the mushroom bodies is linked to diverse roles, such as higher order sensory integration, place memory, motor control, visual navigation, learning and memory (e. g. Farris, 2005; Margulies et al., 2005; Strausfeld et al., 1998).

The ability of the brain to integrate information from different senses is also important for *vertebrates* when detecting, localizing and identifying external events and mediating the responses to these signals. There are many studies that examined multisensory integration in vertebrates, including humans in physiological, behavioural and psychological experiments. Since my study is mainly concerned with the blowfly, I will only briefly touch this wide field of research in vertebrates. The interactions between sensory systems can be beneficial in situations where an animal receives information about an object provided by different sensory systems, for instance when a predator obtains visual and acoustic sensory cues about a prey (or *vice versa*) (recent review: Bulkin and Groh, 2006). For instance, behavioural experiments in barn owls using acoustic and visual stimuli revealed that the animals produce head saccades with the shortest reaction time and the greatest accuracy to audiovisual cues when compared to the head saccades generated to either acoustic or visual cues in isolation (Whitchurch and Takahashi, 2006). Multisensory integration has not only been shown to mediate faster and more accurate behavioural responses, but also more robust neural responses when compared to responses to unimodal stimulation, (e.g. Goldring et al., 1996; Meredith and Stein, 1983; Wuerger et al., 2003). The neuronal substrate underlying the multisensory integration in

vertebrates is the Superior Colliculus (SC) (Wallace et al., 1996). The responses of bimodal SC-neurons to a cross-modal stimulus combination cannot only exceed the largest of the unisensory responses, but can also exceed their arithmetic sum (Rowland et al., 2007). On the other hand, multimodal stimuli that are significantly discordant (e.g. stimuli from different locations) can have the opposite effect and depress responses (Deutschländer et al., 2002; Populin and Yin, 2002; Stein et al., 1989).

Differences in cruising flight behaviour between male and female blowflies

Because chasing is male-specific behaviour, the present study is mainly concerned with males. However, the majority of previous studies on flight behaviour of flies were conducted on females. In my study some statements, for instance, regarding the saccadic characteristics of cruising flights, are based on the conclusions of studies done with female flies. These statements may be critical if important parameters of flight behaviour differ between male and female flies. One way to find out sex-specific differences may be to analyse the components of flight behaviour in both sexes. However, an established comparison is complicated on freely flying flies. When flies freely fly in a flight arena as employed in my study, the actual visual input and the resulting behavioural responses strongly depend on the fly's particular behaviour, as is especially the case during chases and optomotor responses. Two studies that used flies *(Syritta*, *Musca)* of both sexes investigated the optomotor responses under free-flight conditions and reported no sex-specific differences (Collett, 1980a; Srinivasan and Bernard, 1977). The optomotor following response as is exhibited by male *Lucilia* in my study is very similar to the optomotor response as described in females in other fly species. Furthermore, the neuronal substrate that is thought to subserve optic flow processing, the so-called tangential cells (TCs), has previously been described as sexually isomorphic in blowflies (Hausen and Egelhaaf, 1989). The TCs in the lobula plate are assumed to be tuned to different aspects of optic flow (see chapter 2.2.4; reviews: e.g. Borst and Haag, 2002; Egelhaaf et al., 2005; Hausen and Egelhaaf, 1989; Krapp, 2000).

A comparative analysis of flight behaviour between sexes cannot be based on chasing behaviour, because female flies do not perform chases. In cruising flights, the flies do not follow any obvious visual stimulus, the perception of which may be influenced by the fly's particular behaviour. Thus, I conducted two sets of experiments done with male and female *Lucilia,* respectively, that were freely cruising inside the flight arena, and I tested whether there are sex-specific differences in the cruising behaviour using the parameters forward velocity, yaw velocity and turning frequency. I found that the flight patterns during cruising flights are quite similar in both sexes, with the only exception that, on average, male blowflies execute saccades with higher yaw velocities than females (Fig. 3.10). It is not clear whether and to which degree this result is influenced by the properties of the flight arena used in my study. While male blowflies (*Lucilia*) reached peak

turning velocities of up to 3000°/s in this flight arena, female blowflies (*Calliphora*) flying in a flight arena of comparable size exhibited maximum angular velocities of the order of 2000°/s (Schilstra and van Hateren, 1999). Female *Calliphora* were found to reach peak turning velocities of up to 4000°/s in other environments (Kern, personal communication). I cannot explain these differences in peak yaw velocities in females of different blowfly species within different flight arenas; however, my findings indicate that within the *same* environment male *Lucilia* show saccades with significantly higher peak turning velocities than female *Lucilia*. Currently, I can only speculate about an explanation.

Presumably these differences in saccadic turning behaviour between male and female blowflies denote a further evidence of a male-specific feature, such as the chasing behaviour. Sex-specific differences in flies are not only known on behaviour, but also on physiology. Many studies demonstrated that within the visual system of male flies there are structural and physiological sex-specific specialisations at several levels that are assumed to be advantageous to chasing behaviour (see chapter 2.2.1; Burton and Laughlin 2003; Francescini et al. 1981; Gilbert and Strausfeld, 1991; Gronenberg and Strausfeld, 1991; Hardie et al. 1981; Hornstein et al. 2000; Land 1997; Land and Eckert 1985; Strausfeld, 1991). Moreover, it was recently found that in hoverflies, one of the HS-cells in the lobula plate is sexually dimorphic (Nordström et al., 2008). The three HS (Horizontal System) cells are thought to provide input to the optomotor system, which is assumed to exist in male and female flies (see chapter 2.2.4). The so-called HSN-cell has in male hoverflies a substantially smaller receptive field compared to females. The receptive field is confined to the fronto-dorsal visual field which is associated with specialisations of visual pursuit. In addition, this HSN-cell in male hoverflies responds vigorously to small targets against background, suggesting that under certain conditions this neuron might participate in the target signalling pathway. Furthermore, specific behavioural response components are attributed to the activity of particular muscles (Egelhaaf, 1989). The use of different flight muscles in male flies as compared to females may allow the males to perform faster flight manoeuvres than females.

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4 Characterisation of a blowfly male-specific neuron using behaviourally generated visual stimuli

4.1 INTRODUCTION

Males of several fly species pursue potential mates or rivals in visually mediated high-speed aerobatic chases during their courtship behaviour (e.g. Collett and Land 1975; Collett 1980; Land 1993a, b; Land and Collett 1974; Wagner 1986a, b, c; Wehrhahn 1979; Wehrhahn et al. 1982; Zeil 1983). To catch a female fly the male fly has to detect the potential mate and then follow it by adjusting his speed and direction of locomotion to the course of the target. Eventually – if successful – the chase will culminate in a catch of the target and – if the target turns out to be a female conspecific – in copulation (Wehrhahn 1979; Wagner 1986a, b, c). The chasing behaviour of male flies belongs to the fastest visually controlled behaviours that can be found in nature. How does the underlying neuronal substrate of the visual pathway in the fly's brain achieve both pursuit control and successful capture of conspecific females which are both mandatory prerequisites for mating?

Behavioural experiments with male blowflies chasing each other have led to phenomenological models of the control system underlying chasing behaviour (Land and Collett 1974). According to this model the visual cues used for chasing control are the target position on the retina of the pursuer and the retinal target velocity. During aerial pursuit the chasing fly minimizes deviations of the target's retinal position from the frontal midline by yaw rotations. Land and Collett's model was extended when it became clear that dummy targets of different sizes are followed in a systematically different way (Boeddeker et al. 2003). To this end an extra visual pathway where the retinal target size controls the forward velocity of the pursuer was added. In numerical simulations of chasing behaviour the proposed control system generates qualitatively the same behaviour as real blowflies. (Boeddeker and Egelhaaf 2003; Boeddeker and Egelhaaf 2005; Hüls 2005). It is one goal of the study to find out whether this model is implemented at the neuronal level in the visual system of male flies.

My hypothesis is that the specific visual parameters used by the model are separately encoded in different male specific neurons. I test this hypothesis for one of the twelve types of large male-specific visual interneurons (MLG, Male Lobula Giant neuron) that have been identified in the lobula of *Calliphora* and *Sarcophaga* (Gilbert and Strausfeld 1991; Strausfeld 1991). These male-specific neurons (MLGs) are most likely the neuronal substrate mediating chasing behaviour (e.g. Gilbert and Strausfeld 1991; Hausen and Strausfeld 1980; Wachenfeld 1994). MLGs terminate on descending neurons, which descend to the thoracic ganglia and have connections

with neck and flight motor neurons (Gronenberg and Strausfeld 1991). Regarding physiology, the MLG1 neuron is best investigated so far: it exhibits pronounced direction selectivity, its receptive field subtends the area of the acute zone, and it responds well to small moving objects (Gilbert and Strausfeld 1991; Wachenfeld 1994). Anatomical and physiological characteristics resembling those of MLGs are also described for neurons (STMD, Small Target Motion Detector) found in the lobula of the male hoverfly *Eristalis* (Nordström et al. 2006).

Other sexual dimorphisms at several levels of the visual system seem to be uniquely adapted to chasing behaviour and are believed to increase the male's abilities to catch females. In male calliphorid flies the medulla, which is the second neuropile along the visual pathway, has more columns than in females, and the lobula and lobula plate (i.e. the third optic neuropile) are larger in males than in females (Strausfeld 1991). During pursuit male flies fixate their target in the frontal region of the retina (e.g. Boeddeker et al 2003; Collett 1980; Land and Collett 1974; Land 1993; Wagner 1986b; Wehrhahn 1979; Zeil 1983). This eye region has been called 'acute zone' and has structural and physiological characteristics that provide the photoreceptors in the acute zone with high spatial resolution and fast and reliable responses (Burton and Laughlin 2003; Francescini et al. 1981; Hardie et al. 1981; Hornstein et al. 2000; Land 1997; Land and Eckert 1985). The receptive fields of MLG1 and nine other MLG neurons cover the acute zone.

Recent studies indicate that taking the natural stimulus conditions into account can be essential for an understanding of the functional relevance of neuronal computations (e.g. Boeddeker et al. 2005; Burton and Laughlin 2003; Kayser et al. 2004; Kern et al. 2005; Reinagel 2001; Simoncelli 2003; Simoncelli and Olshausen 2001; van Hateren 1997; van Hateren et al. 2005). To this end I combined behavioural and electrophysiological experiments. This allowed me to use dynamical stimuli, as they are experienced by the male fly during chases to analyse the characteristics of a blowfly male-specific neuron for chasing behaviour.

To obtain naturalistic stimuli I recorded with high speed cameras chases after dummy targets moving on circular trajectories. The retinal size and the angular position of the target were reconstructed (Boeddeker et al. 2003) and replayed to male flies, while recording the electrical activity of MLG1. To facilitate comparison of my results to those obtained in previous electrophysiological studies I additionally recorded the responses of MLG1 to experimenter-defined stimuli. Thus, the main goals of this study are (1) to characterise the response properties of MLG1 on the basis of naturalistic visual stimuli and (2) to test whether specific stimulus parameters are encoded by MLG1.

4.2 MATERIALS AND METHODS

Preparation

The electrophysiological experiments were performed on 12h – 2-day old male blowflies (*Calliphora vicina*). Before dissection, the animals were briefly anaesthetised with CO2 and mounted ventral side up on a small glass plate by applying wax to abdomen and the wings. The head was tilted forward and fixed with wax to the thorax. Legs and antennas were removed and the wounds were sealed with wax to prevent the animal from drying-up. The head capsule was opened from behind; air sacs and fat tissue were removed. To prevent movements of the brain caused by the peristaltic movement of the gut the proboscis was cut away and the gut was pulled out and fixed to the thorax with wax. The fly's head was aligned with reference to the symmetry of the deep pseudopupil (Franceschini and Kirschfeld 1971). For the electrophysiological experiment the fly was mounted onto a heavy recording table, facing the monitor.

Electrophysiology

I recorded electrical responses from the axon or from the primary dendrite of the MLG1 in the right half of the brain. The ground electrode, a blunt glass electrode was filled with Ringer's solution (containing in mM: NaCl 128.3, KCl 5.4, CaCl2 1.9, NaHCO3 4.8, Na2HPO4 3.3, KH2PO4 3.4, glucose 13.9, pH 7.0; all chemicals were from Merck, Darmstadt, Germany) and placed on the left half of the brain. To keep the tissue moist Ringer's solution was supplied to the brain by a syringe connected to the ground electrode holder. For intracellular recording glass capillaries (GC100TF-10; Clark electromedical instruments, Pangbourne, UK; outer diameter 1 mm) were pulled on a Flaming/Brown Micropipette Puller (Model P-97, Sutter Instrument Company, Novato, CA). The tip of the recording electrode was filled either with a saturated solution of the fluorescent dye 6-carboxy-fluorescein (Molecular Probes, Eugene, OR) dissolved in 1 M potassium acetate, or with a 12 mM solution of Alexa 488 hydrazide (Molecular Probes) made as follows: 1 mg Alexa 488 was dissolved in 60 µl 5mM KOH; subsequently 20 µl of this Alexa/KOH solution were dissolved in 30 µl 0.25 M KCl giving the 12 mM Alexa solution. The shaft of the electrode was filled in the latter case (Alexa KOH/KCl) with 0.25 M KCl and otherwise (6-carboxy-fluorescein) with 0.2 M potassium acetate.

The electrodes had resistances between 40 and 120 Mega Ohm. The recorded signals were filtered (low-pass: 2 kHz), amplified by the use of standard electrophysiological equipment, and fed into a computer through the analogue input of an I/O-card (DT 3001, Data Translation) at a rate of 4 kHz. For identification the neuron was iontophoretically filled with fluorescent dye by applying a negative current of 1-2 nA to the recording electrode. After the experiment, the fly's brain (whole mount) was viewed through a fluorescence microscope (Leitz, Wetzlar, Germany) and the filled cell was photographed *in vivo* with a digital camera. Only cells, which could be unambiguously identified on the basis of prior neuroanatomical studies (Gilbert

and Strausfeld 1991; Strausfeld 1991; Wachenfeld 1994), were used for further analysis.

High-speed video analysis of chasing behaviour

Chasing behaviour was analysed, on the one hand, to characterise the visual stimuli experienced by the chasing fly during aerial pursuit and, on the other hand, to use a selection of such sequences for visual stimulation in electrophysiological experiments.

Male blowflies (age: 2-7 day) were released in a cylindrical flight arena (91 x 50 cm). Black painted glass spheres (diameter: 8.3 and 13 mm) served as dummy flies and were moved counter-clockwise on a circular track (radius: 10 cm) in a horizontal plane. The speed of the spheres ranged between 1 and 1.5 m/s, which resides within the speed range of real flies. The side walls of the arena consisted of a white drapery, the ceiling and the bottom were homogeneously white, and the arena was illuminated from outside by nine 50W halogen lamps. The temperature ranged between 25°C and 35°C. Male flies either chasing the dummy or a real fly were filmed with two orthogonally arranged high speed cameras (MotionPro, Redlake, San Diego, CA. Sampling rate: 500 Hz; spatial resolution: 1024 x 1024 pixels). One camera was positioned besides and the other below the arena, viewing the centre of the arena through holes of the wall. Video sequences were stored as uncompressed 8-Bit AVI-files on computer hard disk for off-line processing. The 2-D position and longitudinal body axis orientation of the objects were determined frame by frame with the aid of custom-built software, using standard image processing algorithms (Lindemann et al. 2003). Knowing the relative position of the two cameras, it is possible to transform 2-D image coordinates into an orthographic 3-D coordinate system (e.g. Boeddeker et al. 2003; Zeil 1983). I determined for each frame of the video the 3-D position and the yaw orientation of the chasing male fly and the position of the target. Furthermore, I estimated in every frame the fly's horizontal gaze direction from its yaw body orientation. Although blowflies can move their head (Hengstenberg 1993; Land 1973), rotations of the head relative to the surroundings about the pitch and roll axes are generally small during flight (Schilstra and van Hateren 1998) and yaw head rotations are usually in phase, though somewhat faster than yaw body rotations (van Hateren and Schilstra 1999). Since the head movements could not be resolved in the behavioural data, the same type of head-body coordination was assumed in chasing behaviour as characterised during spontaneous flight. Therefore, I did not simulate these rotational degrees of freedom, but set both the roll and pitch orientation of the chasing fly to 0° and assumed that the yaw angle of the head was aligned with the body long axis. The reconstruction of the 3-D-trajectories and all further data processing was done in MATLAB.

Visual stimulation

The position of stimuli is given by the coordinates ψ and θ, denoting the horizontal

and vertical angular positions of the stimulus-centre with respect to the longitudinal axis of the head (Fig. 4.1). $\psi = 0°$ and $\theta = 0°$ are defined by the cross-sections of the horizontal eye equator with the vertical symmetry plane of the eye. Positions $\theta > 0°$ and $\theta < 0°$ are in the dorsal and ventral visual hemisphere, respectively; positions $\psi > 0°$ and $\psi < 0°$ are in the right and left hemisphere of the visual field, respectively.

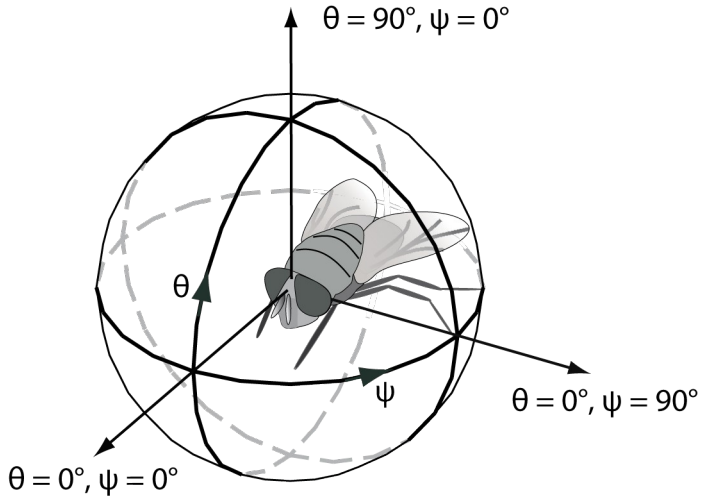

Figure 4.1 Polar coordinate system of the fly's visual field. A particular position in the visual field of the fly is specified by the angles of azimuth ψ and elevation θ. The position $\psi = 0°$, $\theta = 0°$ is given by the cross-sections of the horizontal eye equator with the longitudinal body axis, $\psi = 0°$, $\theta = 90°$ is given by the cross-sections of the horizontal eye equator and the vertical body axis, and $\psi = 90°$, $\theta = 0°$ is given by the cross-sections of the horizontal eye equator and the lateral body axis.

For visual stimulation, two types of stimuli were displayed: experimenter-defined ('artificial') stimuli and behaviourally generated ('naturalistic') stimuli. Stimuli were presented on a monitor screen (Tektronix 608, Tektronix, Wilsonville, OR), which was positioned in front of the fly covering the fly's visual field from $\theta = -10°$ to $\theta = 60°$ vertically and from $\psi = -40°$ to $\psi = 40°$ horizontally.

Artificial stimuli consisted of a square dot or a bar moving with a constant velocity of 180°/s. Dots sized 5° x 5° moved either upward or downward between $\theta = -10°$ and $\theta = 60°$, and clockwise (i.e. left-to-right) or counter-clockwise (i.e. right-to-left) between $\psi = -40°$ and $\psi = 40°$ along different vertical or horizontal paths within the visual field. The horizontal positions of the three vertical motion traces were $\psi = -20°$, 6°, 25° and the vertical positions of the three horizontal motion traces were $\theta = 4°$, 22°, 58°. The bar extended over the entire monitor screen and had a size of 80° x 5° (length x height) when presented horizontally, or 5° x 70° (length x height) when presented vertically. The bar either moved upward or downward between $\theta = -10°$ and $\theta = 60°$, and clockwise or counter-clockwise between $\psi = -40°$ and $\psi = 40°$. Artificial stimuli were programmed in MATLAB (The Mathworks, Natick, MA), assigned to the stimulation software, transferred to the image synthesizer and displayed on the monitor (for details see below). Stimulus size and velocity refer to

the centre of the screen.

Naturalistic stimuli where presented as a dark spot that moved over the homogeneously bright monitor according to the stimulus parameters reconstructed from behavioural data. For technical reasons I had to approximate the form of the target by a black square. Scripts for visual stimulation and data acquisition were written with DT Measure Foundry (Data Translation, Marlboro, MA). Two Data Translation I/O-cards (DT 3001) were used: one for recording neuronal activity and the other for controlling the visual stimulus. Output signals were sent through the analogue channels of one I/O-card to an image synthesizer (Picasso, Innisfree, Cambridge, MA; frame rate: 200 Hz) and were displayed on a Tektronix cathode ray tube monitor (Tektronix 608, Tektronix, Wilsonville, OR). The pattern contrast was 82% (Michelsen contrast) and the mean luminance of the monitor was 31 cd/m^2 measured with a luminance meter (LS 100, Minolta, Osaka, Japan). The control signals of target size and position as well as the membrane potential of the neuron were sampled at a rate of 4 kHz and stored for further analysis.

One run of the visual stimulation protocol consisted of nine different stimuli in a fixed sequence with a pause of 3 s between each stimulus: five behaviourally generated naturalistic stimuli and four artificial stimuli. The naturalistic stimuli consisted of one reconstructed chase with a fly chasing another fly and of three reconstructed chases with a fly chasing the dummy target. The naturalistic stimuli lasted between 300 ms and 2500 ms depending on the duration of the corresponding chase. The duration of artificial stimuli was 1500 ms. The stimulus protocol was presented repetitively, as long as the intracellular recording was stable (up to 18 min.).

Data analysis

I subtracted the resting potential from each individual response trace and calculated the mean over all response traces to each stimulus of each cell. Responses were smoothed using a Savitzky-Golay Filter (span 39, polynomial degree 8; Orfanidis 1996; build-in function of MATLAB-Toolbox) to eliminate high-frequency fluctuations due to noise and active membrane properties. In addition, the mean over the cells that fulfilled the quality criteria (see below) was calculated. For the data analysis of the naturalistic stimuli, the responses were normalised to the maximum amplitude of the potential measured during the stimulations. Data analysis was performed after compensating for the latency between stimulus and response (30 ms).

For motion-sensitive visual neurons, the movement of contrast edges rather than of homogeneous areas is assumed to contribute to the neuronal response. I determined the local motion sensitivity of MLG1 to naturalistic stimuli by analysing the relationship between every single motion step of the stimulus edges and corners – represented by their velocity vectors – and the corresponding membrane potential.

The velocity vectors and the corresponding neuronal responses were calculated with a resolution of 5 ms, which is the interframe interval of the stimulus monitor. Since the neuronal responses were sampled at 4 kHz, this procedure required time averaging over 20 data points. For further analysis, motion vectors were classified with respect to their velocity and their location in the fly's visual field, and the mean vector and its standard deviation were calculated for three different classes of neuronal responses: weak, moderate and strong (details see Results). This procedure allowed me to separate local motion vectors that lead to strong responses from those that do not match the cell's tuning and thus lead to weaker responses.

To quantify the variability of neuronal responses, I calculated the signal-to-noise ratio (SNR) in five MLG1 recordings in different flies. The time-dependent stimulus-induced response component (i.e. signal) was determined as the average of all individual responses across trials. The time-dependent noise component of the responses was determined by calculating the standard deviation of each individual response trace from the ensemble average. The SNR was then obtained as the ratio of the time-dependent signal and the time-dependent noise component. To compare this ratio of MLG1 cells to neurons with well-characterised response properties, I calculated the SNR from recordings of five motion-sensitive HS-cells (Horizontal System cells) (e.g. Hausen 1982a, b) and two motion-sensitive VS-cells (Vertical System cells) (Hengstenberg 1982; Hengstenberg et al. 1982; Krapp et al. 1998). Although these motion-sensitive cells are thought to be involved in detecting self-motion of the animal, they are known to respond to a single moving spot (Krapp et al. 1998), which allowed us to use the same stimulus for stimulating MLG1 and HS- and VS-cells.

Coherence analysis was used to test a possible representation of specific stimulus parameters in the neuronal response. An integral part of the coherence analysis is the reverse reconstruction technique (Haag and Borst 1998; van Hateren and Snippe 2001): To obtain an estimated (reconstructed) time-dependent stimulus from the measured response, the time-dependent neuronal response is convolved with the linear temporal filter that minimises the difference between the real stimulus and the reconstructed stimulus. The coherence function gives a measure of the similarity between the real and the reconstructed stimulus for different frequencies. The values of the coherence vary between 0 (i.e. both signals are unrelated and/or the system is corrupted by noise) and 1 (i.e. linear relationship). Given a linear system that is corrupted by additive noise, the 'expected coherence' is the coherence between single responses and a noise-free system response. The latter is approximated by averaging many responses and the result can be considered as the response that the best possible (non-linear) model should give (Haag and Borst 1998; van Hateren and Snippe 2001).

4.3 RESULTS

Recordings from 10 MLG1 cells were examined for recording quality (signal strength, signal shape), for systematic drifts in the resting membrane potential and for quality of dye-fill (identification). Most recordings lasted only several minutes. Only five recordings from five different flies lasted sufficiently long for testing the entire stimulation programme while also meeting the recording quality criteria. After dye filling the recorded cells, I identified MLG1 anatomically in a whole mount preparation (Fig. 4.2). Since the MLG neurons have already been characterised anatomically before (Gilbert and Strausfeld 1991; Gronenberg and Strausfeld 1991; Strausfeld 1991; Wachenfeld 1994), I refrained from a three-dimensional reconstruction.

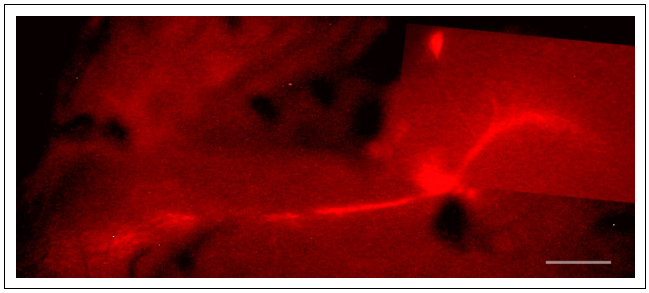

Figure 4.2 Anatomy of MLG1. Photomontage of an MLG1 neuron as seen in a whole mount preparation after staining the cell with the fluorescent dye Alexa Fluor 488. The dendritic arborisation subtends the upper frontal part of the lobula (*right part of the figure*), and the axon passes to the contralateral side of the brain (*left part of the figure*, compare Fig. 2.10). *Scale bar:* 250µm.

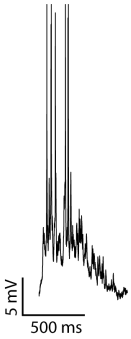

Figure 4.3 Electrical responses of MLG1. Single response of an MLG1 neuron to a bar moving upwards (details see Methods). The response is plotted for the entire duration of bar motion.

Receptive field organisation of MLG1

To be able to relate the characteristics of MLG1 obtained with naturalistic stimuli to conclusions drawn previously, I analysed the activity of this cell type with a sample of experimenter defined ('simple') stimuli similar to those employed more systematically in earlier studies (Gilbert and Strausfeld, 1991; Gronenberg and Strausfeld, 1991; Wachenfeld, 1994). I did not attempt to replicate these studies in detail, since my results with experimenter defined stimuli qualitatively agreed with the earlier findings. These results will, therefore, be summarised only briefly.

MLG1 neurons respond to motion with a graded shift in membrane potential, often superimposed with spike-like depolarisations, so-called spikelets (Fig. 4.3). The membrane potential depolarises by up to 20 mV and spikelets may have amplitudes of up to 25 mV. To find out which visual cues induce robust responses in MLG1, I determined the average over all response traces to a given experimenter-defined stimulus and over five cells (see Methods). A 30 ms latency between stimulus and response was estimated as the time delay between the onset of depolarisation in response to motion of a horizontal bar (80° x 5°) moving upwards along the vertical eye axis and the actual onset of bar motion. This value is only an estimate because the exact border of the receptive field is unknown. However, 30 ms corresponds to the value of MLG1 latency obtained in a previous study by measuring the depolarisation onset in response to dot motion stimuli starting at different positions within the receptive field and moving in the four orthogonal directions (Wachenfeld 1994). For the analysis of input-output relations the responses traces were shifted by 30 ms to compensate for the latency between stimulus and response.

In accordance with previous studies (Gilbert and Strausfeld 1991; Strausfeld 1991; Wachenfeld 1994) the receptive field of the MLG1 neuron is located in the dorsofrontal visual field and covers the acute zone of the eye. MLG1 is sensitive to motion of the dot in different directions: Depolarisations to upward motion, i.e. preferred direction motion, are measured nearly all-over the dorsofrontal part of the visual field and are strongest around θ=4° and ψ=6°. Examples of response averages of MLG1 neurons to experimenter-defined stimuli are shown in Fig. 4.4. During downward motion (i.e. null-direction motion) the cells are either not depolarised or weakly (0.5–3mV) hyperpolarised. Furthermore, depolarisations occur for horizontal motion directions, i.e. for clockwise and counterclockwise motion. Looking at the neuronal responses in detail (Fig. 4.4), one can see that the direction selectivity varies within the receptive field: The depolarisations during clockwise motion occur mainly for ipsilaterally presented dots (i.e. ψ>0°), whereas depolarisations for counterclockwise motion occur mainly contralaterally (i.e. ψ<0°). Upward motion of a horizontal bar leads to even larger response amplitudes than upward moving dots (Fig. 4.4A). Bars moving downwards evoke, like dots, weak hyperpolarisations. Vertical bars moving horizontally induce, contrary to dots, either no or weak depolarisations (Fig. 4.4A).

The lacking or only weak hyperpolarisations obtained during null-direction motion are surprising given the pronounced hyperpolarisations during null-direction motion of another class of motion sensitive cells of blowflies, the tangential cells (reviews see Borst and Haag 2002; Egelhaaf et al. 2002; Egelhaaf et al. 2005; Hausen and Egelhaaf 1989). Whereas in tangential cells the ratio between depolarisation and hyperpolarisation during preferred and null-direction motion was found in a range 1:0.6 and 1:0.8 (Hausen 1982b; Hengstenberg 1982; Kurtz et al. 2001), the ratio in MLG1 is only 1:0.15.

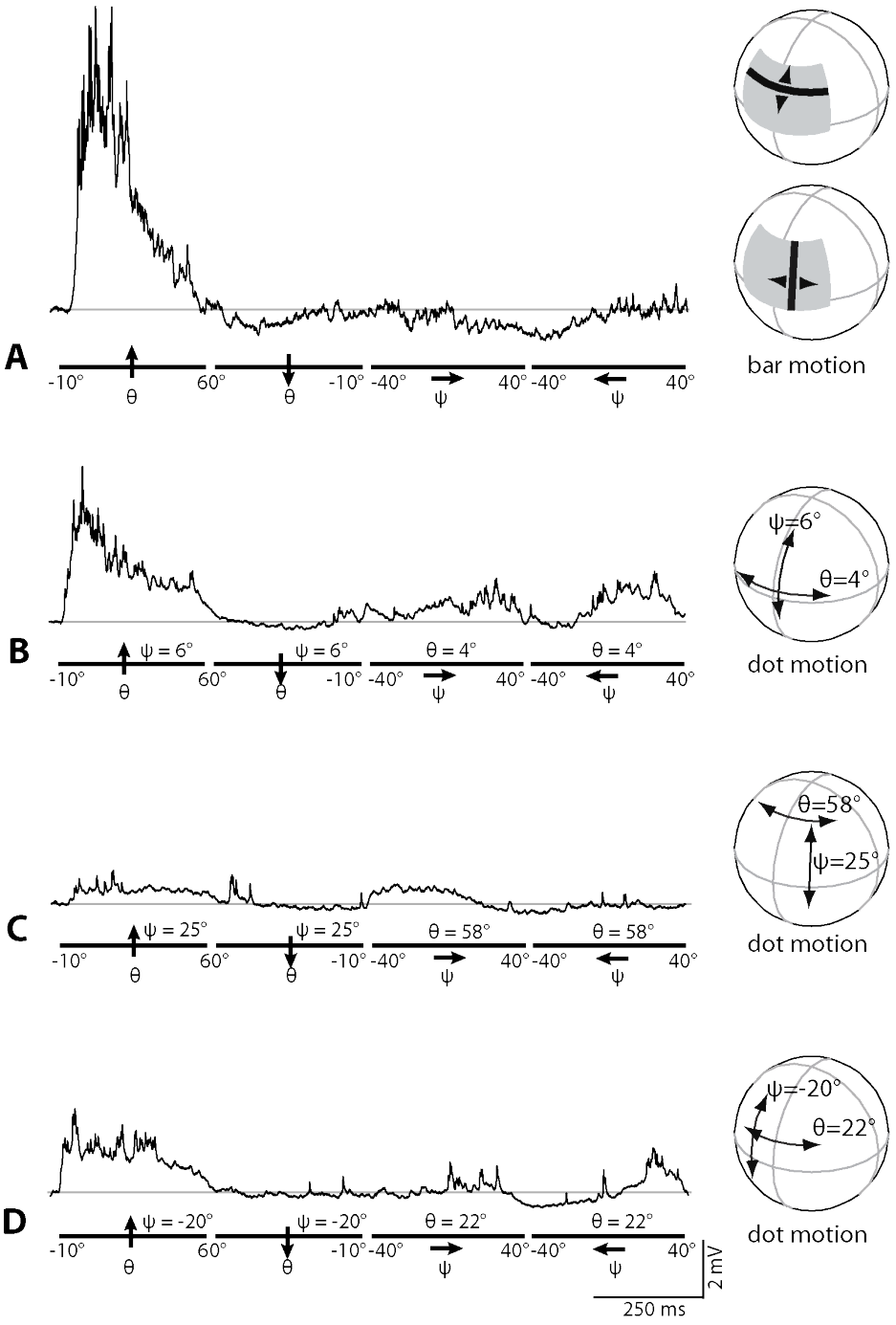

Figure 4.4 Responses of MLG1 to experimenter-defined motion stimuli. A spot or a bar was moved with a constant velocity of 180°/s subsequently along different vertical or horizontal tracks. The *grey horizontal line* denotes the resting potential. *Black lines* beneath response traces denote the motion duration; the *arrow* denotes the motion direction (details see Methods). **(A)** Average of five single response traces to a horizontal and vertical bar moving vertically and horizontally, respectively. **(B)** Average of 22 response traces to a spot moving at ψ = 6° up and down and at θ = 4° clockwise and counter-clockwise. **(C)** Average of 22 response traces to spots moving up and down at ψ = 25°, clockwise and counter-clockwise at θ = 58°. **(D)** Average of 22 response traces to spots moving up and down at ψ = -20°, clockwise and counterclockwise at θ = 22°. ***Insets*** Stimulus traces and areas are plotted onto the polar coordinate system of the fly's visual field to illustrate the actual positions and extend of stimuli. For clarity, stimuli, traces and areas are not drawn to scale.

MLG1 responses to naturalistic stimuli

To find out to what extent the responses of MLG1 to natural motion stimuli can be explained on the basis of their receptive field properties and direction selectivity as determined by conventional experimenter defined stimuli, I reconstructed what the chasing fly had seen during chasing manoeuvres. Therefore, I reconstructed the trajectories of three male flies chasing after dummy targets that moved on a circular track (two examples are shown in Fig. 4.5) and of one male fly chasing after another male (trajectory not shown). In the example with a dummy speed of 1.2 m/s the chase was terminated with a catch after about 310 ms (Fig. 4.5A). With a somewhat faster dummy speed (1.5 m/s) the male fly continued to pursue the target for 2.5 s without succeeding to catch it (Fig. 4.5B). Because of the limited recording times it proved impracticable to use data from more chases in electrophysiological experiments. It should be noted that the visual input of the chasing fly is more complex when it chases another fly than a dummy: the dummy target moved at constant speed in a horizontal plane, whereas chases of another fly usually have a complex three-dimensional structure. The reduced, but still high complexity of the input during dummy chases facilitated establishing stimulus-response relationships for behaviourally generated visual stimuli.

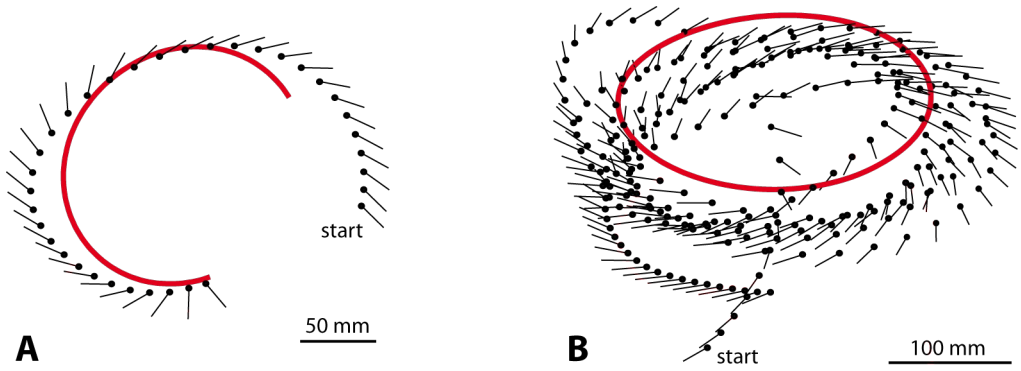

Figure 4.5 Reconstruction of three-dimensional flight trajectories of two chasing flights. **(A)** The reconstructed flight trajectory of a male fly chasing the target (*red line*), which moved on a circular track in top view. The fly is indicated by the position of its body centre (*dot*) and the orientation of the body length axis (*line*). The *start* position of the fly at the beginning of the chase is indicated. The temporal resolution is 20 ms. **(B)** Long pursuit of the target without capture in a view from an oblique angle; same plotting conventions as in (A). The flight in (A) is shown from above; the flight in (B) is shown from an oblique direction.

At the beginning of the chase, the pursuer is distant and, hence, the size of the dummy is small (Fig. 4.6A). During the course of the flight the retinal target size increases and tends to be coupled with an increase of the target's vertical position. When the fly draws closer to the target, the retinal image motion will thus contain mainly upward components. The target's vertical and horizontal positions are confined within a restricted range of the fronto-dorsal visual field, as is particularly apparent for long chases (Fig. 4.6B; see also Boeddeker et al. 2003). In any case,

during chasing of a dummy and of real flies, the visual stimuli are characterised by simultaneous variation of several visual parameters over time. To analyse the responses of MLG1 to naturalistic stimuli, I replayed the reconstructed retinal image sequences on a monitor while simultaneously recording the neuronal activity. When stimulated with these types of naturalistic stimuli, the MLG1 neuron shows strong depolarisations at several instances in time (Fig. 4.6i, ii).

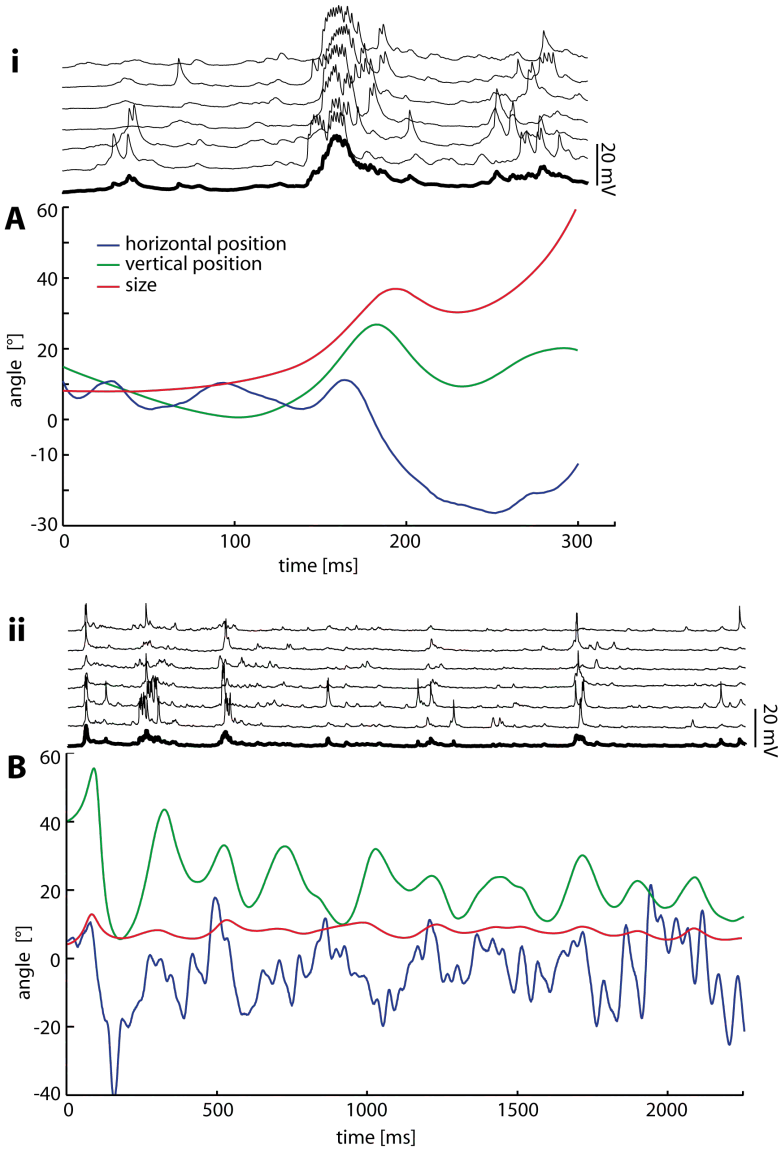

Figure 4.6 Reconstructed time-dependent variation of the target's size and position on the retina of the pursuer of two chases and responses of MLG1. **(A)** The *red line* denotes the retinal size, the *blue and green lines* denote the centre of the target's horizontal and vertical position respectively as experienced by the male fly during the chase shown in Fig. 4.5A. During approach (between about 100 ms – 200 ms and between about 240 ms – 300 ms) the target's elevation and size tend to increase simultaneously. **(i)** Single response traces (*thin lines*) and the average over the six single traces (*bold line, bottom trace*) to the stimulus. **(B)** Same variables obtained from the chase shown in Fig. 4.5B. During the chase, the target is well fixated within the acute zone; the target's vertical and horizontal position resides mainly within θ = 10° – 30° and ψ = -20° – 20°, which corresponds to the most sensitive part of the receptive field of MLG1. **(ii)** Single response traces and the average over the six single traces to the stimulus. Note that these are responses from the same cell as shown in (i).

To illustrate the stimulus situation in the chasing fly's visual field, a section of a motion sequence reconstructed from the chase shown in Fig. 4.5A is projected onto the visual field of the chasing fly (Fig. 4.7A). In this example, the fly initially approaches the target and both retinal target size and elevation increase, leading to an upward movement of the target. Both, upper and lower edges of the target move in the preferred direction of MLG1 within the most sensitive part of its receptive field. The stimulus-induced membrane potential is depicted by the colour of the respective square. During the displayed stimulus sequence, the cell is subject to strong depolarisation as long as both horizontal edges of the target have a strong upward component (Fig. 4.7A, see also neuronal response traces Fig. 4.6i). Note the length of the horizontal edges and the respective high depolarisation level. This is comparable to the above mentioned results with horizontal bars moving upwards inducing maximum response amplitudes (Fig. 4.4).

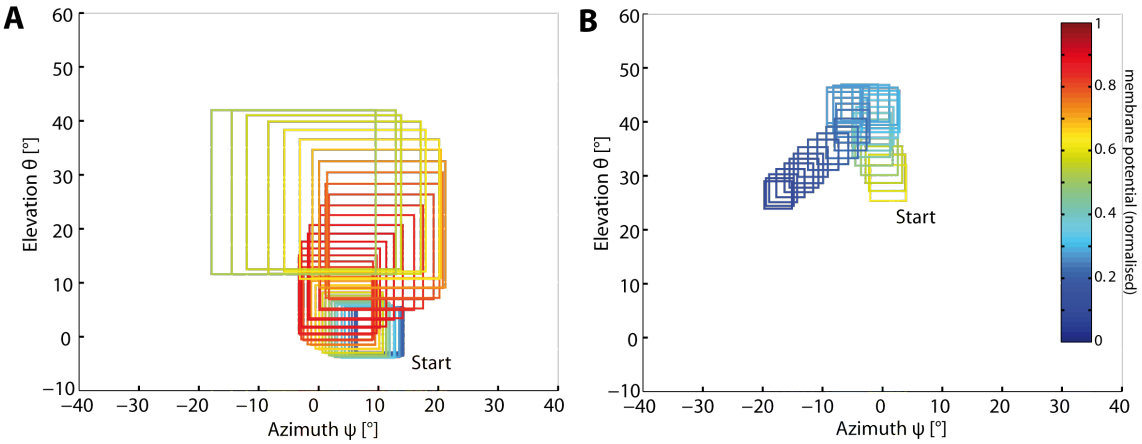

Figure 4.7 Time-dependent motion sequences of the stimulus corresponding to two sections of the chases shown in Figs. 4.5A,B as projected onto the visual field and the corresponding response of the MLG1. For clarity the stimulus (i.e. *squared spots*) is not filled. **(A)** The section shown here corresponds to the time interval between 100–180 ms of Fig. 4.6A. The amplitude of the normalised membrane potential at any time is denoted by the *colour of the respective square* (for the details of normalisation, see chapter 4.2). *Warm colours* denote strong depolarisations, *dark blue* denotes strong hyperpolarisation. The size of the square corresponds to the retinal size of the spot at the respective time. The *start* of the motion sequence is indicated. Edge and corner motion with prevailing upward-components induce a depolarisation, thus revealing the preferred direction of the cell and the approximate centre of its receptive field. The temporal resolution is 2.5 ms. **(B)** The section shown here corresponds to the time interval between 312–430 ms of Fig. 4.6B. Edge motion at the outer parts of the receptive field with mainly downward and horizontal components lead to a repolarisation of the cell. The temporal resolution is 3.75 ms. Same plotting conventions as in (A).

The response amplitude declines when the edges of the target move mainly horizontally towards the outer parts of the receptive field. This is illustrated in Fig. 4.7B, which shows a part of the visual input and the corresponding colour coded responses during the chase shown in Fig. 4.5B: At the beginning, while the horizontal edges of the target move upwards within the most sensitive part of the

receptive field, the response amplitude is moderate, but it continually decreases when the stimulus moves downwards and horizontally or towards the outer parts of the receptive field. Given the shortness of the horizontal edges moving upwards, the respective moderate response levels corroborate those measured to a dot moving upwards (Fig. 4.4).

To quantify the responses to naturalistic visual stimuli and to pool the data of all analysed MLG1 cells I related the response amplitude to the corresponding retinal motion vectors by which the cells were driven. This was done by the following procedure. I divided the stimulated visual field of the fly into equally sized grid elements of 14° x 13.3° (horizontal x vertical size). Since motion-sensitive neurons respond only to the motion of contrast edges of a stimulus pattern rather than to homogeneous areas, I related the MLG1 responses to the motion vectors of the edges and corners of the target. Therefore, I determined for all naturalistic stimuli which edge motion and/or corner motion occurred within each grid element at each instant of time (time resolution 5 ms). The vector length denotes the motion velocity; the vector direction denotes the respective motion direction. To associate the motion vectors with the neuronal response amplitude, the responses were normalised for each cell (see Methods) and segregated into three response classes, 0.67-1 for strong, 0.34-0.66 for moderate and 0-0.33 for weak responses. This represents an acceptable trade-off between the spatial resolution of the grid and the size of the response classes: a fine grid with small elements would include only a small proportion of the MLG1 responses and thus lead to less significant mean vectors; a coarser grid would blur details of local motion tuning over the neuron's receptive field.

For each grid element, all motion vectors associated with a given response class of MLG1 were determined and the mean vector and the standard deviations of its x- and y- components calculated (Fig. 4.8; numbers denote the number of samples contributing to each mean vector). Large neuronal depolarisations are evoked mainly by motion within a region (Fig. 4.8A), roughly corresponding to the centre of the receptive field as determined with simple stimuli. This most sensitive area extends from $\theta = 0°$ to $\theta = 40°$ vertically and from $\psi = -20°$ to $\psi = 25°$ horizontally. However, large depolarisations occur only when the motion vectors contain an upward component, which is in agreement with the cell's preferred direction, as determined with simple stimuli (Fig. 4.4). Mean motion vectors that correspond to moderate response amplitudes and that are evoked in the central region have as well all an upward component. In addition, moderate response amplitudes are measured at the outer parts of the receptive field (Fig. 4.8B). The majority of the mean velocity vectors corresponding to weak neuronal responses are short (Fig. 4.8C). This results partly from the fact that the individual velocity vectors point into a wide range of directions (see large standard deviations), but may also be due to low motion velocities.

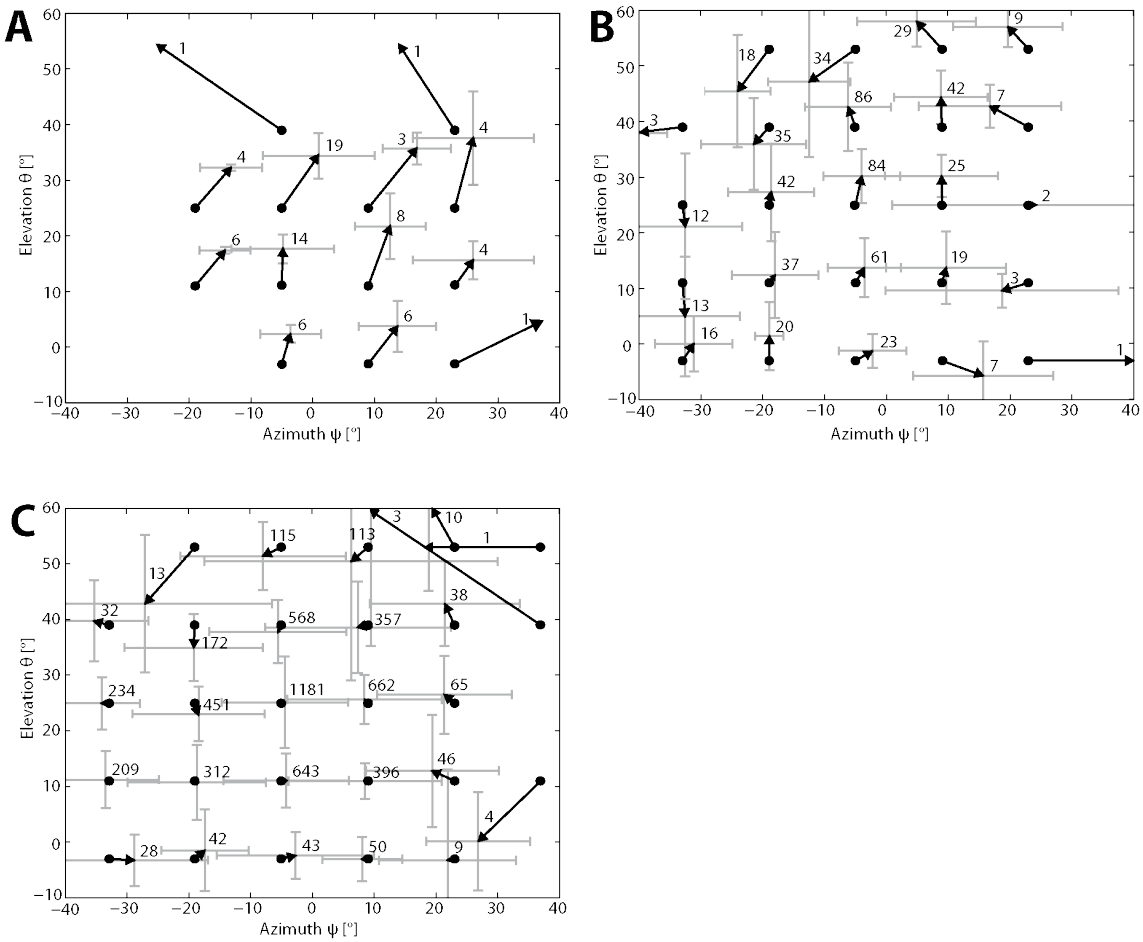

Figure 4.8 Average motion vectors corresponding to large **(A)**, moderate **(B)** and weak responses **(C)** of MLG1. A grid is projected onto the fly's visual field for spatial discretisation. The mean vector that is calculated from the individual motion vectors within each grid element for a given response class (see Methods) is plotted in the centre of the grid element. The *vector length* denotes the motion velocity; the *vector direction* denotes the motion direction. The *starting point* of the motion vectors corresponds to the centre of the respective grid element. The *cross* at the vector head denotes the standard deviations of its x- and y-components. The *number of samples* contributing to the mean vector is in most cases depicted in the first quadrant of its standard deviation cross bar. For clarity, *arrowheads* of short vectors are small. The size of the arrowheads is not of significance. **(A)** All mean motion vectors corresponding to large responses include an upward component. **(B)** The cell exhibits moderate responses to upward motion within the central part of the receptive field, to horizontal motion and to motion at the outer parts of the receptive field. **(C)** For weak responses the corresponding mean motion vectors are short, the number of samples is high. The large standard deviations indicate that individual motion vectors are considerably different in direction and length.

Within the most sensitive region of the cell's receptive field, preferred-direction motion may induce strong, but also moderate or even weak depolarisations. Usually, the responses of motion sensitive neurons depend on stimulus velocity. Which image velocities occur during real chasing situations? I grouped the motion velocities to three classes: low velocities: 0° – 180°/s, medium velocities: 180° – 500°/s and high velocities: 500° – 2000°/s. During chases, edge and corner velocities of 180° – 500°/s occur frequently (47%), whereas higher and lower velocities occur in only

29% and 24% of cases respectively. To test whether this feature may affect MLG1 responses, I selected for each response class and each grid element only those responses which were elicited by motion with a strong upward component (i.e. pointing upwards θ: 90°,±65°). The responses were then attributed to one of three velocity ranges (low velocities: 0° – 180°/s; medium velocities: 180° – 500°/s; high velocities: 500° – 2000°/s) and the frequency of occurrence was determined (Table 1). Large responses are only evoked by high and medium velocities, moderate responses predominately by medium velocities, and weak responses almost exclusively by low and medium velocities. These results indicate that during stimulation with naturalistic motion stimuli, the MLG1 neurons exhibit a broad velocity-tuning with preferences for velocities higher than 180°/s.

Table 1. Relative frequency of occurrence (in percent) of different response amplitudes for the different classes of motion velocities including the velocity vectors located within the centre of the receptive field and pointing θ: 90° ± 65° upwards.

	Velocities 0-180°/s	Velocities 180-500°/s	Velocities 500-2000°/s
Large responses (%)	0	40	60
Moderate responses (%)	2.1	81.6	16.3
Weak responses (%)	39.7	55.3	5

The number of samples for the three velocity classes (0° – 180°/s, 180° – 500°/s, 500° – 2000°/s) are 416, 844 and 141 respectively.

There might be another stimulus feature affecting the response amplitude, i.e. the duration of motion in a particular direction. One striking feature of the visual stimuli occurring during chasing manoeuvres is a frequent change in stimulus direction (Fig. 4.6). An edge of the target moving within the most sensitive part of the receptive field for only 5 – 10 ms in the preferred direction may induce a weaker neuronal depolarisation than a target that moves there for 50 ms. Therefore, I determined the time intervals within which an edge moved more or less continuously upwards (θ: 90°,±65°) within the centre of the receptive field of MLG1 at velocities above 180°/s. The longer an edge moves with a preferred parameter constellation in the receptive field centre, the higher are the evoked response amplitudes (Table 2).

Table 2. Dependence of neuronal response amplitudes on motion duration.

	Mean motion duration in preferred direction (ms)	Standard deviation of the motion duration (ms)
Large responses	42.5	3.5
Moderate responses	20.6	12.9
Weak responses	10.9	8.9

The mean motion duration gives the average time within which an edge moved with a range of directions centred about upward motion ($\theta = 90° \pm 65°$) in the centre of the receptive field and at velocities > 180°/s. The membrane potential is assorted into one of three response classes. The number of samples for the class of high, moderate and weak responses is 2, 8 and 28 respectively.

In conclusion, the responses of MLG1 to naturalistic chasing stimuli depend on direction, velocity, duration of motion and on the position of motion within the visual field. These results are in accordance with the motion sensitivities and receptive field properties of MLG1 as characterised with simple stimuli.

Variability of MLG1 responses

The MLG1 neuron is likely to play a role in the extremely fast chasing system. Contrary to expectations that this cell should respond reliably during chasing manoeuvres, the individual responses to the same naturalistic motion sequence show a high degree of variability (Figs. 4.6i, ii). To put the variability of MLG1 cells into the context of neurons with well-characterised response properties, I recorded motion-sensitive tangential cells (five HS- and two VS-cells) while presenting the same stimulus protocol as was used for characterising MLG1 (data not shown). Both cell classes responded quite well. The variability was quantified by comparing the signal-to-noise ratio (for details see Methods). For HS-cells, the signal-to-noise ratio is 2.3, for VS-cells 2.0 and for MLG1 neuron 1.7. Thus, the variability between single responses to the same stimulus is slightly higher for MLG1 cells than for VS- and HS-cells, although the naturalistic stimuli characteristic of chasing situations that were used in the experiments are not the optimal stimuli of the latter two cell types.

Are retinal target size and position encoded by MLG1?

Previous behavioural experiments led to the conclusion that the chasing control system relies to a large extent on two input parameters: the retinal size and position of the target (Boeddeker et al. 2003; Boeddeker and Egelhaaf 2003; Boeddeker and Egelhaaf 2005). Therefore, I wanted to test, based on naturalistic stimuli, whether

the MLG1 neuron encodes exclusively one of these visual input parameters and calculated the coherence between the neuronal responses and any of these parameters (Fig. 4.9). The coherence is a frequency-dependent measure that allows me to assess how well the time course of a particular stimulus parameter (here: retinal position or retinal size) can be reconstructed by a linear filter from the neuronal responses (for details, see section 4.2). The coherence varies between 0 (i.e. both signals are unrelated, no reliable reconstruction possible) and 1 (i.e. perfect reconstruction). The coherences between the neuronal responses and the retinal size as well as x- and y-position of the target, respectively, are relatively small, indicating that neither of these stimulus parameters is encoded reliably by MLG1.

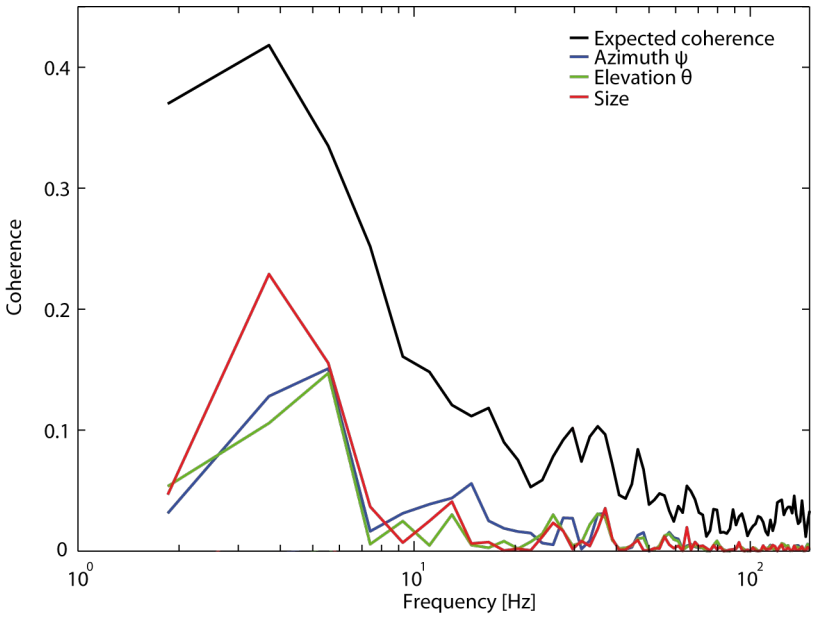

Figure 4.9 The coherence between three visual parameters and the neuronal responses as well as the expected coherence are shown. I find small coherences for the retinal size as well as for x- and y-position of the target, respectively. Though each of the individual parameters may be somehow represented in the responses of MLG1, there is no explicit linear dependence of the neuronal responses on distinct parameters. The expected coherence can be considered as an upper bound of the coherence function of the system. The deviation of the expected coherence from 1 is induced by the noise of the neuronal responses.

Since a small coherence can have its cause in very noisy neuronal responses as well as in a non-linear relationship between the responses and the analysed stimulus parameter, I also determined the expected coherence. The expected coherence is a measure of neuronal variability and represents the best performance that could, in principle, be obtained for the given noise level of the cell. The expected coherence is considerably smaller than 1 even at low frequencies, corroborating the above conclusion that MLG1 responses reveal a high variability. Moreover, for all tested visual parameters the coherence values are much smaller than the expected coherence. This indicates that each of the individual parameters is somehow represented in the responses of MLG1; however, I have no explicit linear

dependence of the neuronal responses on distinct stimulus parameters.

4.4 DISCUSSION

In the present study, I characterised a male-specific visual interneuron (MLG1) of the blowfly *Calliphora* using naturalistic visual stimuli, as they occur during real chases and tested the hypothesis of a possible representation of specific visual parameters in the neuronal responses.

Characterisation and receptive field properties of the MLG1 neuron

MLG1 responds with graded shifts in membrane potential, often superimposed by spike-like depolarisations and shows a distinct direction selectivity, which varies within the receptive field. The cell responds best to visual motion stimuli that contain upward components in the dorso-frontal area of the visual field, which is in accordance with previous studies (Gilbert and Strausfeld 1991; Wachenfeld 1994). MLG1 exhibits no pronounced inhibition during null-direction motion which suggests that the mechanism underlying direction selectivity differs in this neuron from the mechanisms of motion detection characterised in tangential cells (e.g. Egelhaaf and Borst 1993). The analyses with naturalistic stimuli reveal the following MLG1 characteristics: (1) sensitivity to several visual parameters (motion direction, position within the visual field, motion velocity and duration of motion) and their variation over time and (2) rather complex response characteristics to the joint occurrence of multiple visual parameters. Because the response characteristics of MLG1 as obtained with naturalistic stimulation corroborate the results found with simple stimuli, pronounced nonlinear interactions of the more complex stimulus parameters under natural conditions do not appear to strongly shape MLG1 responses.

MLG1 has a large receptive field that is located in the dorsofrontal region of the retina, thus covering most of the retinal area where the target is fixated during pursuit. MLG1 responds well to stimuli that extend beyond the frontal edge of the visual field of the eyes into the contralateral visual field (Beersma et al. 1977; Wachenfeld 1994). My findings confirm earlier conclusions that MLG1 does not only receive input from the ipsilateral eye, but also from at least one contralateral neuron. This input may be conveyed to MLG1 by the axon of the contralateral MLG1, since the cell has been suggested to be dye-coupled and thus, possibly electrically coupled with its contralateral counterpart (Gilbert and Strausfeld 1991; Wachenfeld 1994). Additionally, a coupling with the contralateral counterpart could be one way to realise the motion sensitivity of the MLG1 in the other half of the visual system (see Fig. 4.4).

Male-specific characteristics of the visual system

Chasing behaviour is characterised by extreme virtuosity (Land and Collett 1974; Wehrhahn 1979; Wehrhahn et al. 1982; Zeil 1983; Wagner 1986b, c; Land 1993a, b; Boeddeker et al. 2003). Therefore, the pursuit control system can be assumed to work very fast, efficiently and reliably. The male-specific 'acute zone' has structural and physiological qualities of the visual system which seem to be uniquely adapted for chasing behaviour and should increase the male's ability for successful chasing: Within the acute zone, male *Calliphora* and *Syritta* show larger facets and lower interommatidial angles, which are indicative of higher sensitivity and acuity (Land and Eckert 1985; Land 1997). Photoreceptors within an ommatidium are normally differentiated into achromatic (six receptors, i.e. R1–R6) and chromatic (two receptors, i.e. R7 and R8) pathways. However, within the acute zone of male *Musca*, not only photoreceptors R1–R6, but also R7 and R8 contribute to the achromatic pathway (Franceschini et al. 1981; Hardie et al. 1981). This increased convergence of photoreceptors on second order neurons is believed to improve the detectability of dark targets. Furthermore, male acute zone photoreceptors in *Musca* respond more strongly than female photoreceptors to moving targets (Burton and Laughlin 2003). Additionally, in *Musca*, the acute zone photoreceptors show a higher spatial resolution and much faster electrical responses than female photoreceptors, thus allowing encoding higher velocities and smaller targets (Hornstein et al. 2000). Altogether, these characteristics may induce both improvement of the SNR and shortening of the latency of MLGs.

Latency, variability and small-field selectivity of the MLG1 neuron

Although chasing behaviour is extremely fast, the 30 ms latency of MLG1 estimated in this study is not lower but in the range of other motion sensitive fly neurons, e.g. the H1 neuron (Warzecha and Egelhaaf 2000). This is in accordance with a previous study (Wachenfeld 1994). I did not try to determine the exact latency of the MLG1 neuron, which is very likely to depend on stimulus contrast and on room temperature (Warzecha and Egelhaaf 2000). To test whether the conclusions drawn in the present paper depend on the exact latency value I also tested latencies of 20 and 25 ms. I found that the results are largely independent of the exact latency value because I obtained qualitatively the same results for shorter latencies.

At first sight, a neuronal latency of 30 ms appears to contradict time lags of 20 ms as estimated for the control of yaw velocity in chasing behaviour (Boeddeker et al. 2003). However, the behavioural time lag was estimated by cross-correlating the time-dependent angular position of the target and the yaw velocity. In my study the time lag was estimated by a different method i.e. as the delay between the onset of a bar moving in the preferred direction of the cell and the onset of depolarisation in the neuronal response. Moreover, the temporal resolution in the behavioural study was limited by the interframe interval of 20 ms and could thus well be slightly shorter or longer than 20 ms. It should be noted that the time lag for the control

system of forward velocity is 60–80 ms and thus larger than that of yaw control and of the MLG1 neuron.

The variability of MLG1 responses is surprising, being not lower, but even slightly higher than the variability of tangential cells stimulated with the same stimuli. Despite this variability, 'optimal' stimuli induce a robust and strong depolarisation of MLG1. Moreover, I cannot exclude that MLG1 operates faster and more reliably than in my electrophysiological experiments when the fly is actually flying and that the responses become more reliable with the age of the fly. For technical reasons, my electrophysiological experiments had to be done on very young flies, i.e. at a age where the males would not yet chase females.

As expected for a neuron presumably tuned to small moving objects, MLG1 responds well to moving spots, but depolarisations induced by a bar moving upwards are stronger than responses to a spot moving in the same direction. This finding is in contrast to a previous study in which bars of differing width were used to stimulate MLG1 and the smallest object induced the largest response amplitudes (Wachenfeld 1994). This discrepancy may be due to differences in the details of the stimuli used in the two studies, but can currently not be resolved. However, with regard to horizontal motion, MLG1 responds better to small moving spots than to moving bars. Recently, neurons in the lobula complex of the male hoverfly *Eristalis* have been characterised as small target motion detectors (STMD). These are sharply tuned to small (0.8°x3°) moving targets and some STMDs are even inhibited by large objects (Nordström et al. 2006). Neurons tuned to small objects are not specific to insects, but have also been found in the visual system of vertebrates. For instance, directionally selective neurons in the tectofugal system of birds respond to target motion (object diameter 1°) and are inhibited by whole-field motion as may occur during self-induced motion (Frost et al. 1990). In this regard the bird neurons are similar to the MLG1 neuron of blowflies and STMD neurons of hoverflies.

The role of MLG1 within a pursuit controller

What may be the role of MLG1 in visually guided behaviour? Model simulations based on behavioural experiments showed that a control system with retinal size and position as input variables can account for many features of the chasing behaviour of blowflies (Boeddeker and Egelhaaf 2003; Boeddeker et al. 2003). Furthermore, the image velocity of the target was assumed to improve fixation control during chasing (Land and Collett 1974; Land 1992; Boeddeker and Egelhaaf 2005). A possible way to implement this control system may be a neuronal substrate with parallel pathways encoding the target's retinal size, position, and velocity. From my present knowledge it appears likely that MLG1 takes part in a target fixation control system (Gilbert and Strausfeld 1991) without exclusively signalling target size or position. I employed a coherence analysis, to find out how well retinal target size or target position can be reconstructed on the basis of the neuronal responses. I could not confirm the hypothesis of an explicit representation of either

the retinal size or the position of the target in MLG1 responses. Rather, size, position and velocity and their variation over time jointly affect the responses of MLG1. So far, I have not taken into account the other (at least 11) male specific neurons. It is plausible to assume that a chasing controller may employ the whole ensemble of specified neurons and that these may play different roles in encoding the dynamic visual input during chasing behaviour. The division of the control system into distinct pathways, exclusively signalling separate visual target parameters may thus be convenient for analytical reasons, but seems to get blurred at the neuronal level.

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