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Optomotor course control in flies with largely asymmetric visual input

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Abstract We have studied freely flying and walking flies as well as flies flying in a flight simulator in order to discover how functionally blinding one of the eyes affects the fly's ability to move straight. It is hard to tell just by observing the animals' movements whether they have been deprived of vision in one eye. Statistical analysis is need to show that there are differences in the locomotory paths of monocular and binocular flies: monocular flies tend to turn slightly towards the side of the seeing eye. It is possible that the superimposed translational and rotational optic flow fields, generated on the trajectory of monocular flies, sum to zero net flow. This overall flow over the retina of the open eye might lead to a state of optomotor equilibrium.

Key words Vision · Optic flow · Optomotor behaviour · Course control · Fly

Introduction

Usually we take it for granted that animals including ourselves are able to move on a straight course. How does an animal know that it is moving straight ahead? If the motor system were symmetrically organised and if there were no external disturbances, there would be no reason why moving on a straight path should be a problem at all. In real life, however, external disturbances occur frequently, and the motor system cannot be expected to be organised perfectly symmetrically. Sensory cues are need for course stabilisation. Vision, for example, is concluded to play a prominent role in moving straight. This conclusion can be drawn from the observation that humans can walk straight in the presence of visual landmarks while they are reported to walk on large-radius circular tracks under conditions of poor contrast, i.e. when it is foggy, or in environments like deserts that have little visual structure (Schaeffer 1928).

There are various ways to obtain the relevant information about the direction of motion from vision, i.e. from the continual retinal image displacements which are called 'optic flow'. One simple possibility is to compare the overall retinal image motion as is experienced by either eye. If the animal moves on a straight path in an uniformly structured environment, the optic flow on both eyes should be roughly the same. Accordingly, the activity of corresponding neurons in both halves of the visual system exploiting the optic flow should be approximately equivalent. The animal would then be in a state of optomotor equilibrium (review: Wehner 1981). Large differences in the activity of such neurons would, in contrast, indicate asymmetric optic flow as is likely to occur during deviations from a straight course. If the animal intends to move straight, these asymmetries are assumed to be used to control corrective steering manoeuvres to regain a state of optomotor equilibrium and, thus, the intended straight course.

Indeed, for the optomotor system of the fly there is evidence for such a scheme from both behavioural and electrophysiological experiments. The fly is a well-analysed model system for investigating the neural mechanisms underlying the processing of optic flow and its role in visual orientation (recent reviews: Egelhaaf and Borst 1993a; Egelhaaf and Warzecha 1999). The yaw torque and the thrust responses elicited by visual stimuli simulating rotational and translational motion of the animal, respectively, could be explained most parsimoniously by assuming that the spatially pooled motion signals originating from the two eyes are compared by some sort of subtraction at the level of the motor system (Götz 1968, 1975). A similar conclusion has been drawn for visual position stabilisation of the hummingbird hawk moth (Kern and Varjú 1998). On the basis of electrophysiological recordings from a range of fly

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steering muscles involved in mediating turning responses of the animal, a mirror-symmetrical input organisation of the flight motor has been suggested. Moreover, the different steering muscles have been shown to be activated in a directionally selective way by the pooled motion signals from large parts of one or both eyes (Egelhaaf 1989; review: Heide 1983).

If the ability of an animal to move on a straight course were indeed due to a comparison of the overall optic flow on the two eyes, one might expect severe consequences for this ability after occluding one of the eyes. Indeed, in many animal species circular movements have been described under such experimental conditions (e.g. Fraenkel and Gunn 1961). However, our own observations of free-flying flies with one eye blinded did not indicate that the animals behave unusually. This observation prompted us to analyse systematically the orientation behaviour in the blowfly *Lucilia* when vision is monocular and thus to unravel mechanisms which the fly might use to move straight. The experiments were done with different behavioural paradigms, i.e. by video analysis of the trajectories of freely flying and walking flies, as well as by recording the yaw torque of tethered flying flies in a flight simulator. The analysis with freely moving animals comes relatively close to natural conditions of locomotion. The analysis in the flight simulator allows a more systematic study of input-output relationships because the visual stimuli can be manipulated more easily by the experimenter. Despite these differences in the experimental approach, the results obtained with both types of behavioural paradigm are surprisingly similar. Course control of monocular animals is only weakly affected as compared to controls: on average, trajectories of partially blinded animals deviate slightly towards the seeing eye.

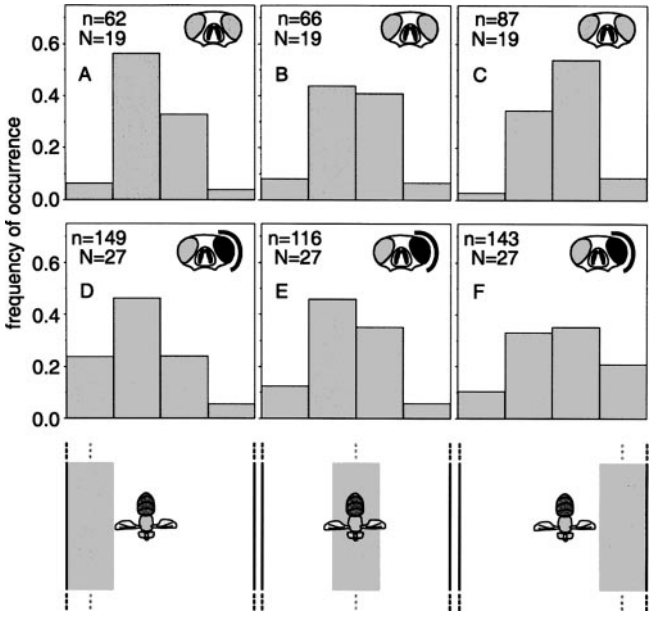

Fig. 1A–F Experiments on flies flying in a tunnel (*insets bottom row*). Control flies (**A–C**) and monocularly blinded animals (**D–F**) flew from one end of the tunnel to the other, with an axial floor pattern either centred between the side walls (**B,E**) or shifted to the right (**A,D**) or left (**C,F**) wall, respectively. Experiments were performed on both animals with the left or right eye blinded, respectively. Data from both groups were pooled and are shown as if obtained exclusively with animals seeing with the *right* eye only (*insets* **D–F**). About half way through the tunnel the horizontal position of the fly within the tunnel was determined and assigned accordingly to one of four classes. Each class represents a 10-cm-wide vertical section of the tunnel. Figures show the relative number of flight trajectories belonging to each class. Control flies flew in the middle of the tunnel when the floor pattern is centred (**B**). Trajectories are shifted to the side of the floor pattern in (**A**) and (**C**). Monocularly blinded animals behave quite similarly (**D–F**). However, the flight trajectories tend to be shifted slightly to the open eye. *n* number of trials, *N* number of animals participating

Materials and methods

All experiments were done on female blowflies of the genus *Lucilia*. The animals came from our laboratory stock which, to avoid in-breeding, we refresh several times a year with animals caught in the wild.

Free-flight experiments

At least 2 days before an experimental series started, 10–15 animals were monocularly blinded under light CO_2 anaesthesia using red or black nail polish to coat one of the eyes. A similar number of control flies was treated in the same way, but water was used instead of nail polish. After the experiments, careful inspection of the blinded eyes ensured that only data from flies that were still properly blinded were analysed.

The experiments were done in a wooden box (height 0.4 m; width 0.4 m; length 2.3 m). Only the end wall and the top wall of the box were made of mesh wire (thickness 0.1 mm, mesh width 0.7 mm) to allow video recording of the flight trajectories. In the front wall there was a small hole through which the flies were introduced into the arena. The front wall, the side walls, as well as the floor of the arena were homogeneously white. The only exceptions were elongated stripes (length 2.3 m) covered with a random texture consisting of square elements (edge length 2 mm). Two of these horizontally oriented stripes (height 50 mm) were placed symmetrically on either side wall of the arena at a height of 0.27–0.32 m. A third textured stripe (width 100 mm) was placed on the floor of the arena either in its middle or at the right or the left side, respectively (insets Fig. 1). The flies were filmed with two video cameras at a rate of 50 fields s^{-1} (2 fields = 1 frame) both from above as well as from the end of the tunnel. The camera placed above the arena covered the entire width of the arena but only a 0.54-m-long section of its length, starting 0.94 m from the entrance hole (both measured at the top wall of the tunnel). From the video film taken by the camera at the end of the flight tunnel, both the height and the lateral position of the fly in the tunnel were determined when it crossed a reference line (1.2 m from the front wall of the tunnel). The top view was used to estimate the velocity of the fly. Animals were not marked but tested individually repeatedly. Potential temporal effects of blinding on the locomotory behaviour were not investigated.

Video-analysis of walking trajectories

The general procedures for preparing the animals were as for the free-flight experiments. In addition, the wings of all animals were cut close to their base to prevent them from flying away. All animals were kept in the dark until about 20 min before an experiment started.

The experiments were done in a cylindrical arena (radius 0.25 m; height 0.295 m). The walls of the arena were covered with a random texture consisting of square elements with a side length of 2 mm. The floor of the arena was homogeneously white. Flies were

released individually into the arena from below through a central hole. The arena was illuminated from above. The light originating from four halogen lamps (500 W each) was reflected from a large screen made of polystyrene placed above the arena. The luminance at the floor of the arena was approximately 150 cd m^{-2}. There was a slight brightness gradient along the radius of the arena due to shading effects of the wall. The animals were filmed at a rate of 50 fields s^{-1} from above with a video camera which had access to the arena through a hole cut into the polystyrene screen.

All experiments were done on individually marked flies. Each fly was released several times into the arena. Again, potential temporal effects of blinding on the locomotory behaviour were not investigated. Only those parts of the walking trajectories in which the distance of the fly from the wall of the arena decreased continuously and the fly walked at a minimum forward speed of 30 mm s^{-1} were used for further data analysis. Tracks were discarded from the analysis where the flies stopped walking too frequently and/or for extended periods of time as well as tracks where the flies never reached the wall during the recording time of 120 s. The position and orientation of the longitudinal body axis of the fly was digitised by using commercial software (Global Lab Image, Data Translation) and programmes written by Maik Lutterklas in C/C++ (Borland).

For data analysis the radius of the arena was subdivided into 23 distance classes, starting 30 mm from the centre. The first 21 classes had a width of 10 mm, the last 2 classes had a width of 5 mm. For each trajectory and class the average translational and rotational velocity as well as an angle γ were calculated from the position and orientation of the longitudinal body axis of the fly with respect to an external co-ordinate system. γ is the angle between a line aligned with the longitudinal body axis and the tangent onto the arena wall at the intersection point of the line with the arena wall (inset of Fig. 3C). In the next step of the analysis, for each fly averages of the translational and rotational velocity as well as γ were calculated within the different distance classes. Finally, for each class the median and the first and third quartiles were determined for the flies' average translational and rotational velocities and for the angles γ. Data evaluation was performed with programmes written in C/C++ (Borland).

Experiments on tethered flying flies

Flies were briefly anaesthetised with CO_2. The head was fixed to the thorax with a bridge of bee wax. A small triangular piece of cardboard which served to affix the fly to a torque compensator was glued onto the pronotum. The yaw torque generated by the stationary flying fly was measured with a torque compensator (Götz 1964; Fermi and Reichardt 1963). The torque compensator was operated under closed-loop conditions, i.e. the fly could control by its own yaw torque responses the rotational movement of the visual input around the animal's vertical axis similarly as in free-flight. The torque signal was temporally low-pass filtered with a cut-off at 10 Hz. As in previous studies (Reichardt and Poggio 1976), the angular velocity of image motion was proportional to the fly's yaw torque. A torque of 10^{-7} Nm resulted in an angular pattern velocity of 15.5° s^{-1}.

Visual stimuli, i.e. vertical stripe patterns were generated in a cylindrical arena (diameter 370 mm; height 150 mm) which surrounded the tethered flying fly. The arena consisted of ten identical modules, i.e. arrays of 48 columns and 30 rows of LEDs. The LEDs (5 mm × 2.5 mm, green; type: CQX 11/LTL 6233 LN) had an almost rectangular profile and were soldered in direct neighbourhood on circuit boards. Each module was slightly concave along its horizontal extent to form the cylindrical shape of the arena. Each column of the arrays could be switched on or off independently. The time until an LED reached a constant luminance value after switching amounted to 20–50 μs. It took approximately 370 μs to address all columns serially. The luminance of the bright and dark stripes amounted to 500–900 cd m^{-2} and approximately 20 cd m^{-2}, respectively. The horizontal angular extent of each LED column amounted to 0.75°, its vertical extent to ±22° as viewed from the centre of the arena.

Since the experiments were done under closed-loop conditions, the motion stimuli could not be calculated before the experiments but had to be calculated on-line depending on the fly's actions and reactions. This was done at a rate of 200 Hz, i.e. both the torque signal was sampled and the pattern was generated at this frequency. While the rotational component of the optic flow was controlled by the fly's yaw torque, the translational component of the optic flow was given by the experimenter, since the thrust of the animal could not be measured. In all experiments the tethered flying fly started in the centre of a virtual cylindrical arena. Consequently, all stripes on the walls of the real arena had the same angular horizontal extent of 5.7°. During a pure simulated translation, i.e. when no yaw torque was generated, the pattern moved from front-to-back in both hemifields of the arena. The pole of expansion coincided with the direction of heading of the tethered flying fly. Since in this situation the fly approached the wall of the virtual arena, the stripes in the frontal part of the visual field grew larger, whereas those in the rear part appeared smaller accordingly. The diameter of the virtual arena was 10 m. The fly-controlled rotational component of the optic flow was added to the fixed translational component. The size and position of all stripes of the arena were calculated for each time step at which the stimulus pattern was updated. The programmes for controlling the optic flow under closed-loop conditions and for data acquisition (I/O Board DT 2801 A, Data Translation) were written in C/C++ (Borland) by Bernd Kimmerle. The torque signal was stored for each time step and the position and orientation of the fly in the virtual arena were calculated off-line for further data analysis.

At the beginning of each experiment, the reference torque corresponding to straight flight was determined. In real flight situations this is not a problem at all, since flies generate by definition zero torque as long as they fly straight. However, in the experimental situation flies that are suspended not entirely symmetrically generate a torque even if their muscles are activated in the same way as in free flight. Moreover, there might be asymmetries in the forces generated by the two wings. The reference zero torque in the flight simulator was determined in a five-step process prior to and in the course of an experiment. A constant rotational motion bias was superimposed onto the spontaneous background rotation mediated by the fly's yaw torque response. No translational optic flow was added to these rotations. The direction of the motion bias switched every 5 s. At the beginning of zero torque determination, a fixed arbitrary though plausible value was assumed as reference torque. During successive periods of 10 s the torque generated by the fly as a response to the motion bias in opposite directions was averaged. The average torque was used as the new reference torque for the next 10-s period. The last two reference values were averaged and taken as the reference zero torque for the experiments. This procedure was repeated at least every 30 trials of the experiment. As a criterion of a correct adjustment of the 'zero-torque' level, it was ensured that each fly, on average, flew straight ahead under symmetrical binocular stimulation (data not shown).

Since for zero torque determination the flies had to have binocular vision, it was not possible to occlude one of the eyes with paint. Functional monocularity was therefore obtained by leaving the LED columns in front of one eye dark. Care was taken that the pattern in front of the 'open' eye did not reach the area of binocular overlap. The patterns reached from +(−)18° to +(−)180°, respectively. All trajectories were excluded from the analysis where the torque of the fly was so strong that pattern elements moved by one half of their width or more within one refreshment cycle of the pattern. Hence, trials where aliasing occurred were not included into the analysis.

In the course of an experiment a set of three different visual stimuli was presented repeatedly in pseudo-random order: (1) binocular stimulation, (2) stimulation of the left eye, and (3) stimulation of the right eye. At the beginning of each stimulus presentation (sweep) the fly was in closed-loop with respect to yaw torque, i.e. rotation around its vertical body axis, while no translation of the fly was simulated. Simulation of forward movement started 2 s after the beginning of the sweep. The sweep ended either when the animal came within 0.3 m of the wall of the virtual arena

('successful sweep') or after 20 s had elapsed. Only data from sets with three successful sweeps were analysed.

Three different translational velocities were simulated: (1) 0.5 m s^{-1}, (2) 1 m s^{-1}, and (3) 2 m s^{-1}, covering large parts of the velocity range of freely flying flies. If possible, at least ten data sets per animal and velocity were recorded. Occasionally, however, flies did not co-operate under all three stimulus conditions equally well. Especially at a translational velocity of 0.5 m s^{-1} they frequently stopped flying before the aspired number of data sets was recorded.

The radius of the virtual arena (5 m) was subdivided into 13 distance classes, starting 0.3 m from the centre and ending 0.3 m from the simulated wall. For technical reasons, the simulated approach of a fly towards the wall had to be stopped at this distance. The first 9 classes had a width of 0.4 m, the last 4 had a width of 0.2 m. For each class the median and the first and third quartiles were calculated for the flies' average rotational velocities and for the angles γ in the same way as described above for the walking trajectories.

To check for asymmetries in the experimental setups, experiments of all types presented in this paper were performed on control animals and on animals with the right or left eye functionally blinded, respectively. Data from control animals were evaluated quantitatively and ensured that binocular animals, on average, moved straight (not shown in figures). The data from both monocular groups were pooled appropriately after it was checked that they were approximately mirror symmetrical as is expected due to the bilateral symmetry of the fly. In the figures data are presented as if all monocular animals saw the stimulus with their *right* eye.

Results

Monocular flies in free flight

In order to assess to what extent flies which had one eye occluded ('monocular flies') are able to fly on a straight path, animals were allowed to fly freely in a flight tunnel. Some of the flies only walked on the walls of the tunnel. Others immediately flew through the tunnel towards its other end which was somewhat brighter. Only the latter were included in the data analysis.

Binocular flies tend to fly along the midline of the tunnel if the textured stripe on the floor was in the middle, i.e. the distribution of the fly's position along the transverse axis of the tunnel is approximately symmetrical about its midline (Fig. 1B). When the pattern on the floor was shifted to one of the walls of the tunnel, the distribution was similarly broad but slightly displaced towards the side of the pattern (Fig. 1A, C). This basic finding holds when one eye was occluded. However, monocular flies tend to fly slightly closer to the side of the tunnel next to the seeing eye (Fig. 1D–F). This shift is not statistically significant [χ^2-test according to Brandt-Snedecor (Sachs 1984)]. Indeed, just by observing individual animals flying along the tunnel it is not possible to tell whether or not one eye was occluded. Nonetheless, the overall flight activity of monocular flies was reduced and flight speed was lower (average speed of normal flies: 1.3 m s^{-1}; average speed of monocularly blinded flies: 1.0 m s^{-1}).

Although these experiments clearly show that the ability of flies to fly normally is not much impeded by occluding one of the eyes, they do not allow us to assess what mechanisms the flies use to perform so well. Apart from the optic flow which might affect optomotor course stabilisation, there are various fixation cues, e.g. the edges of the end wall of the tunnel which are seen by the fly for most of the flight in the frontal visual field. Moreover, there was a slight brightness gradient along the axis of the tunnel which proved to attract the fly to fly through the tunnel. Apart from visual cues, the fly has access to mechanosensory information, such as provided by its haltere system. The halteres have been concluded to provide information about body turns of the animal, if these are generated sufficiently fast (Pringle 1948; Nalbach 1993; Nalbach and Hengstenberg 1994). Although such a multitude of sensory cues is the normal case under free-flight conditions in the outside world, we wanted to isolate the optomotor system in order to understand its role in stabilising the course of locomotion and to assess its significance in controlling the course of flies with one eye occluded.

In order to reduce, as far as possible, the potential significance of fixation cues and brightness gradients as well as mechanosensory information from the halteres, further behavioural experiments were performed on (1) flies freely walking in a randomly textured cylindrical arena, and (2) on tethered flies in a flight simulator, apparently flying in a cylindrical arena. Particularly in the latter situation the halteres could not play a role in course control, because the animals did not physically turn, and only the visual consequences of turns were simulated.

Monocular flies, freely walking

When the monocular flies were released in the centre of the cylindrical arena, some of them walked to the wall more or less directly. Others walked seemingly aimlessly in variable directions without reaching the wall within the stipulated 120 s. Many flies only walked intermittently. Others did not walk at all. The same holds true for the control flies. Hence, it was again not possible, just by looking at the individual traces of locomotion, to decide whether or not one of the eyes of the fly was occluded (Fig. 2). In Fig. 3 the median as well as the first and third quartile of the translation velocity and angular velocity are plotted as a function of the distance of the fly from the centre of the arena. Care was taken that the flies started to walk randomly into arbitrary directions. Accordingly, arrival positions of flies at the arena wall are not restricted to certain areas (not shown).

The median translational velocity of the flies was fairly constant over time and basically independent of the distance from the centre of the arena (Fig. 3A). In contrast to the translational velocity, the median angular velocity depended strongly on the distance from the centre. While it was close to zero as long as the fly was close to the centre of the arena, it increased considerably when the fly approached the wall (Fig. 3B). On average, the flies tended to turn towards the side of their

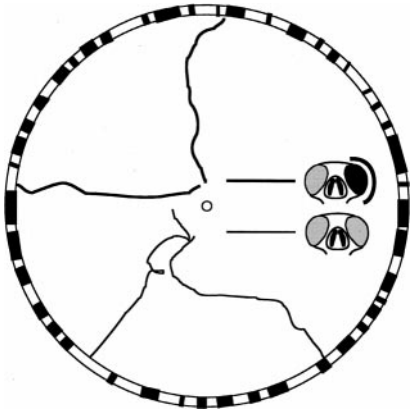

Fig. 2 Examples of walking trajectories of control flies (*thin lines*) and monocularly blinded animals (*thick lines*) approaching the randomly textured wall of a cylindrical arena (diameter 0.5 m). Flies entered the arena from below through a hole in the centre of the white floor. The animals were tested individually; the plot shows superimposed trajectories from different tests

unoccluded eye. This increase in angular velocity was accompanied by changes in the orientation of the fly with respect to the wall (angle γ; see Materials and methods, and inset Fig. 3C for definition). While close to the centre of the arena the fly was oriented almost perpendicularly to the wall, i.e. it walked straight to the wall. The closer it came to the wall, the more the angle between its longitudinal body axis and the wall increased (Fig. 3C).

The average trajectory was reconstructed backwards from the median translational and angular velocities, starting at a distance of 3 mm from the wall at an angle $\gamma = 137.7°$ (Fig. 3D). Polynomials were fitted to the data points in order to obtain values for the translational (7th order) and angular velocities (9th order) at all distances covered by the reconstructed trajectory. Note that close to the wall the angle γ strongly influences the optic flow resulting from a given combination of translational and angular velocity.

In conclusion, although on first sight the walking behaviour of flies is not dramatically affected by occluding one of the eyes, on average, the animals slightly turn towards the open eye. The turning velocity depends on their distance to the textured background.

Monocular flies, flying in a flight simulator

Similar results were obtained with tethered flies flying in the flight simulator. A circular arena was simulated, 10 m in diameter and covered with vertical stripes. At the beginning of each flight, the fly started in the centre of the virtual arena, which means that all stripes surrounding the animal had the same width. In the flight simulator only the yaw torque could be measured and, thus, only the visual consequences of the turns of the animal about their vertical body axis were fed back onto the visual stimulus. The translational velocity had to be simulated.

For all simulated translation velocities, the flies tended to turn towards the open eye. The median angular velocity was small while the fly was still close to the centre of the virtual arena. As was the case for freely walking flies, the median turning velocity increased as the animals approached the wall of the virtual arena (Fig. 4A–C). Interestingly, the angular velocities were larger for larger translational velocities (Fig. 4D). The

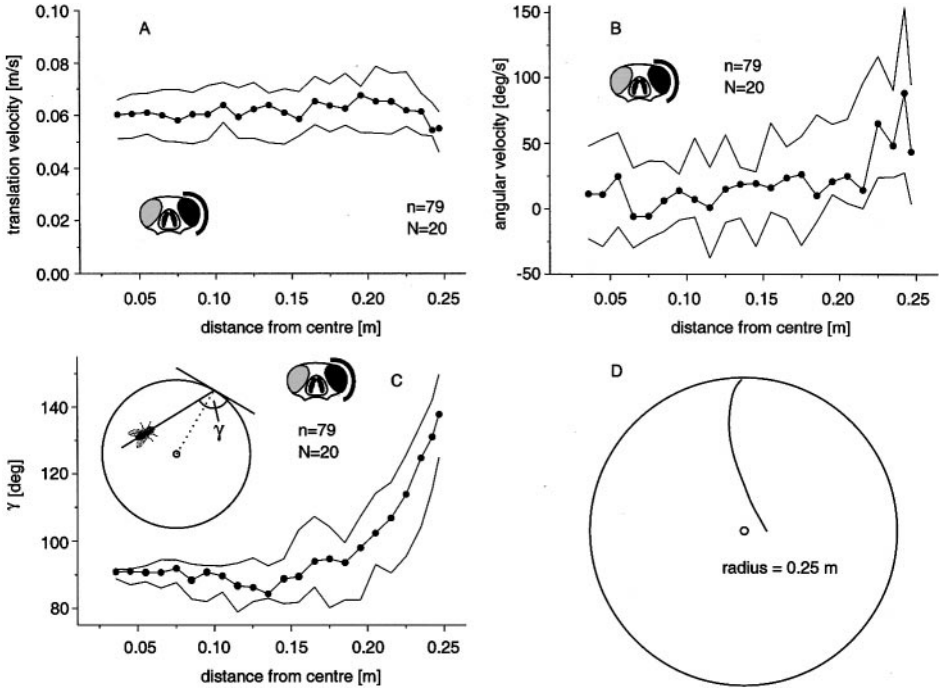

Fig. 3A–D Experiments on walking flies approaching the randomly textured wall of a cylindrical arena (diameter 0.5 m). Either the left or the right eye of the flies was blinded; data are shown as if the *right* eye were open. **A** Median translational velocity; **B** median angular velocity; and **C** median angle γ (for definition see inset and text) within successive distance classes of 10 mm and 5 mm, respectivley, starting 30 mm from the centre of the arena. *Thin lines* denote 1st and 3rd quartiles, respectively. **A** Translational velocity is independent of the distance to the wall except very close to the wall when animals slow down slightly. **B** In contrast, the angular velocity increases with proximity to the wall. **C** This increment is accompanied by increasing γ. **D** Reconstructed average walking trajectory. *n* number of trials, *N* number of animals participating

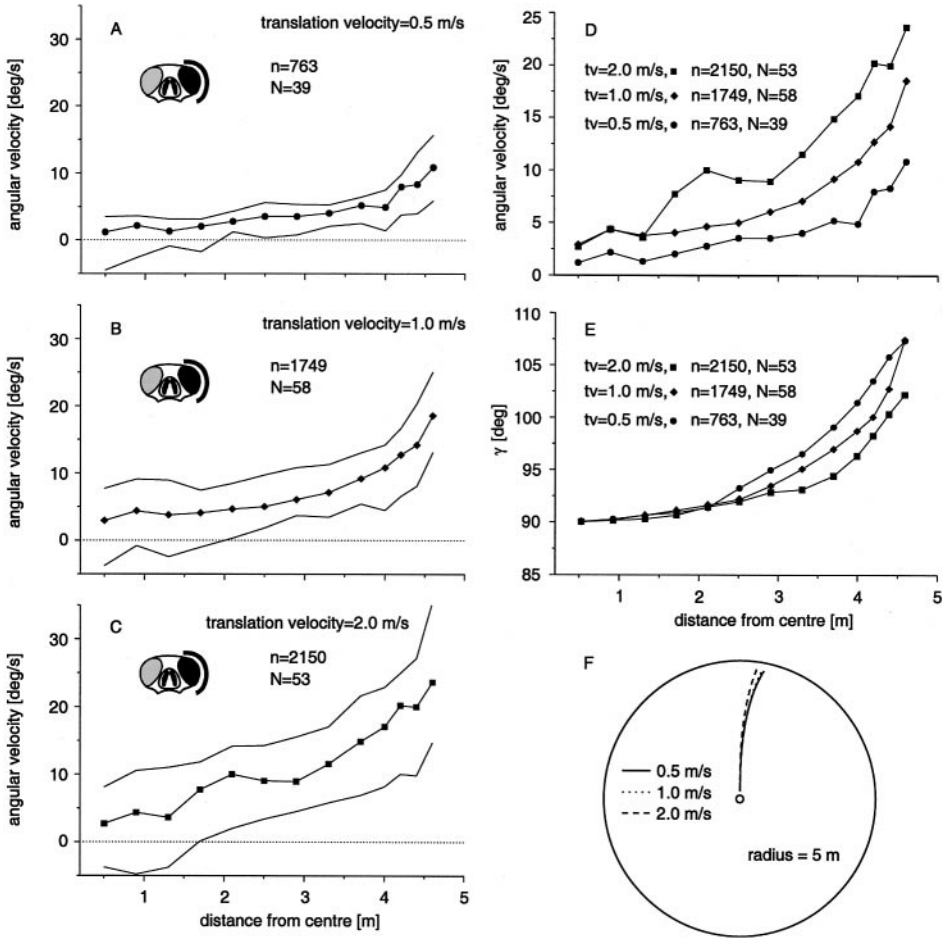

Fig. 4A–F Experiments on tethered flies flying in a flight simulator. The torque of the animals around their vertical axes is measured by a torque meter and controls the horizontal movement of a vertical stripe pattern projected onto the walls of a circular LED arena (closed-loop). In addition front-to-back motion of the pattern is added in either hemifield (open loop) to simulate forward flight of the animal at three different velocities (0.5 m s^{-1}, 1 m s^{-1}, and 2 m s^{-1}). The pattern was either presented left or right; data are shown as if the pattern were presented on the *right*. From the torque signal the rotational velocity and – considering the simulated translation – the angle γ (for definition see inset of Fig. 3C and text) as well as the position within the simulated arena (diameter 10 m) are calculated every 5 ms of the simulated approach to the arena wall. **A–C** The median angular velocity increases with proximity to the wall. The turning tendency is stronger the higher the simulated forward speed. *Thin lines* denote 1st and 3rd quartiles, respectively. **D** Median angular velocities at three different translational velocities (replot of data shown in **A–C**). **E** The increment in angular velocity results in an increment of γ. **F** Reconstructed average flight trajectory. *tv* translation velocity, *n* number of trials, *N* number of animals participating

angle between the longitudinal body axis of the fly and the virtual wall of the arena γ also increased (Fig. 4E), in accordance with the results from walking flies (Fig. 3C). The orientation angle γ was slightly smaller at a given distance to the wall when the simulated translation velocity was large than when the velocity was small (Fig. 4E). Since flies flying with higher simulated translational velocities turn with larger angular velocities than flies flying with lower simulated forward speed, the flies tended to approach the wall of the virtual arena on a similar path (Fig. 4F, average reconstructed flight trajectories).

Discussion

Both freely flying and walking flies can still navigate reasonably well if they are allowed to use only one of their eyes. On the basis of individual tracks of the animals, it is hardly possible to infer whether they had two or only one eye at their disposal. Nonetheless, on average, monocular flies tend to turn towards the stimulated eye. The turning velocity was found to increase with the proximity to the textured wall of the arena as well as with the translation velocity. However, it should be stressed that usually these tendencies cannot be discerned in the individual flight trajectories or walking tracks. Two major complexes of questions arise from these results: (1) what determines the systematic deviations of the averaged tracks of locomotion from a straight course? What sensory cues are relevant and what are the properties of the underlying control system? (2) Why are flies relatively free to deviate from this controlled 'average course'? Before these questions can be answered we need to discuss what sensory cues, both

visual and non-visual, might be available to an animal to infer whether it is moving straight.

Sensory cues that can be used to determine the direction of locomotion

There are both mechanosensory cues as well as visual cues which might tell the animal whether or not it is turning about its vertical axis and thus deviating from a straight course. In humans, for instance, mechanosensory information from both the vestibular system as well as from neck proprioceptors affect the performance of an observer to assess his or her direction of locomotion. However, this mechanosensory information appears to be particularly relevant, when during locomotion the gaze is shifted relative to the direction of locomotion by eye and head movements relative to the body (e.g. Crowell et al. 1998). Otherwise, the direction of locomotion is assumed to be primarily inferred from the characteristic optic flow induced on the eyes during locomotion (Warren and Hannon 1990; Warren et al. 1991).

In flies the situation may be less complicated in this regard, because the eyes are basically immobile in the head capsule and the head can be turned around its vertical axis by not much more than 15° (Hengstenberg 1993). Moreover, there is no evidence that during locomotion flies turn their heads independently from the turns of the whole body. Two principally different ways of body-head co-ordination have been found to operate during turns of the fly about its vertical axis: (1) during voluntary rapid turns of the animal in free flight, the head moves in the same direction as the thorax. The faster head turns start slightly later and finish slightly earlier. Otherwise the head is basically aligned with the longitudinal body axis most of the time (Hateren and Schilstra 1999). (2) When the animal is rotated by an external force, the body rotations are counteracted by compensatory head turns (Hengstenberg 1993). Similar gaze stabilising reflexes operate in walking flies. Here small-amplitude body rotations (3–5°) synchronous with the step cycle of the animal can be observed. The resulting rotatory image displacements are largely reduced by compensatory head turns (Strauss and Heisenberg 1990; Strauss 1991). We therefore conclude that from the reconstructed average walking trajectory one can infer the input of the visual system although the trajectory lacks the body oscillations typical to real individual walking trajectories.

How are body turns and, thus, deviations from straight locomotion detected by the fly nervous system? During walking either the visual system or leg proprioceptors can provide useful information. In flight, a different, specialised mechanosensory system, the halteres yield proprioceptive information (Pringle 1948; Tracey 1975; Nalbach 1993; Nalbach and Hengstenberg 1994) The halteres detect fast turns of the body along all three axes and play a role in mediating compensatory reflexes of the whole animal and the head (Hengstenberg 1993). Likewise directionally selective, motion-sensitive so-called tangential cells (TCs) in the fly's third visual neuropil monitor optic flow as is generated, for instance, during deviations of the animal from its course (Hausen 1981; Hausen and Egelhaaf 1989; Egelhaaf and Borst 1993a; Krapp et al. 1998). Although the significance of TCs in freely moving animals remains to be demonstrated, it is clear from a host of behavioural experiments done on tethered flying flies in flight simulators, that compensatory optomotor turning responses are elicited by motion stimuli that also activate these neurons (for review see Egelhaaf and Borst 1993a; Hausen 1981). Moreover, the compensatory optomotor responses of tethered flying flies are much reduced or even absent, when the respective TCs are missing either in mutants (Heisenberg et al. 1978) or after ablation (Geiger and Nässel 1981; Hausen and Wehrhahn 1983, 1990). Nonetheless, all these experiments do not reveal the significance of the TCs in evaluating optic flow under natural conditions. This is because during free locomotion the dynamical properties and the complexity of optic flow differ considerably from the properties of the optic flow in experiments done in the flight simulator.

Notwithstanding, optic flow helps to straighten the trajectories of locomotion of walking and flying insects. In a textured surrounding, the walking trajectories of insects appear to be less curved than in a homogeneous surrounding (Buddenbrock and Moller-Racke 1952; Wendler and Scharstein 1986; R. Kern, unpublished observations). Similarly, at least the low-frequency rotational components in optic flow are reduced in flies flying tethered in the flight simulator operating under closed-loop as compared to open-loop conditions (Heisenberg and Wolf 1988; A.-K. Warzecha, unpublished results). Hence, visual feedback tends to reduce the rotational component in the optic flow (for review see Collett et al. 1993). Moreover, asymmetries in optic flow in front of the two eyes could be shown to affect the flight trajectories in free-flying bees (Srinivasan et al. 1991) as well as the position in space of the hummingbird hawk moth while hovering in front of a flower (Kern and Varjú 1998).

In order to move straight, only the rotational and not the translational component in optic flow should be compensated for. Hence, it might be advantageous if the neurons evaluating optic flow would respond selectively to either component of optic flow. Interactions between the two eyes can lead to an increased sensitivity to rotational flow relative to translational flow. This computational strategy might be particularly advantageous for animals with lateral eyes and not much binocular overlap. In animals with frontal eyes and much binocular overlap, rotational and translational optic flow might be discriminated almost equally well with one eye. Here, the focus of expansion which is indicative of translation and which plays a role in determining the direction of locomotion from the global pattern of optic flow in some cases can be detected even from the retinal

images of one of the eyes. Indeed, humans are well able to infer the direction of locomotion monocularly (Berg and Brenner 1994a). Moreover, cells have been found in monkey cortical area MST which respond best to image expansion as occurs during translation, i.e. when the focus of expansion is located well within the cells' receptive fields and thus may play a role in detecting the direction of locomotion (e.g. Duffy 1998). Nonetheless, even in humans the detection of the direction of locomotion is improved when depth information based on binocular vision is present (Berg and Brenner 1994b). The detection of the pole of expansion is hardly possible in animals with lateral eyes with only little binocular overlap, since, during translation, the pole of expansion is rather close to the frontal edge of the eyes' visual fields. Hence, a comparison of motion information originating from the two eyes might be advantageous in determining the direction of locomotion. Indeed, interocular interactions have been shown to play an important role in a variety of species, such as in crabs (e.g. Kern et al. 1993), pigeons (e.g. Frost et al. 1994), rabbits (e.g. Simpson et al. 1988), but also in insects (e.g. Kern 1998; Ibbotson and Goodman 1990), including the fly (for review see Hausen 1981; Horstmann et al. 1999). As has already been outlined in the Introduction, the simplest way (at least from a mathematical point of view) to obtain information about the direction of locomotion is to subtract the signals of corresponding analysers of optic flow in the two halves of the visual system. At least in a symmetrical environment, the signals of such elements might cancel each other at a subtraction stage, leading to a state of optomotor equilibrium, thereby indicating that the animal moves straight.

Determinants of the systematic deviations from a straight course in monocular flies

Only visual cues can be responsible for the systematic deviations of the average course of locomotion from a straight course, because the mechanosensory signals which provide information about the direction of locomotion did not change by occluding one eye. In contrast, a state of optomotor equilibrium is no longer given in monocular flies when they move straight. Is it possible that some state of optomotor equilibrium is reached on the average path of locomotion as is observed in both walking and flying flies? This state of equilibrium would still imply cancelling of the signals from the two eyes at the subtraction stage. If the signal from the blinded eye is assumed to be negligible, retinal image motion on the seeing eye should also result in a negligible signal. Otherwise, signals from the two eyes cannot cancel each other at the subtraction stage. By inspecting the optic flow as generated on the seeing eye of the monocular fly while walking on the average path of locomotion, it becomes obvious that this hypothesis may well be correct. Figure 5 illustrates the optic flow on part of the right eye at a given instant of time for four different situations. When a monocular fly moves straight ahead in a cylindrical arena, an optomotor equilibrium can never be reached, since all local velocity vectors point up, down or from front-to-back whereas none points from back-to-front (Fig. 5A). However, the length of the velocity vectors changes with their location in the visual field. Interestingly, a somewhat different walking direction results in a very similar flow field (Fig. 5B). In contrast to translation, when turning on the spot towards the right eye all velocity vectors point in the same direction from back-to-front and, at a given elevation in the visual field, have the same length (Fig. 5C). Therefore, when the animal turns while moving towards the wall of the arena – as is the case on the average path of locomotion of monocular flies – the optic flow contains vectors pointing in all directions (Fig. 5D). Thus, these vectors may well cancel out, when the local motion signals are pooled, leading to a state of optomotor equilibrium in monocular flies.

In principle, an optomotor equilibrium could also be reached by monocular flies if they move on oblique trajectories, depending on the angle between their direction of motion and the body longitudinal axis. Also here the local velocity vectors may point into all directions, allowing cancellation if pooled. However, in the experiments presented here the flies were never observed to walk obliquely.

Whether the cancellation hypothesis is correct, cannot be decided just by calculating the local velocity vectors for the different points in the visual surround, as projected on the fly's retina. The reason for this is, that there is no evidence that local velocity information is represented anywhere in the fly visual system according to its direction and magnitude. Rather the representation of local motion information could be shown to depend also on the textural features of the stimulus pattern, such as its spatial frequency content and contrast as well as on its size (for review see Egelhaaf and Borst 1993b). Moreover, the time-course of the responses of TCs is proportional to pattern velocity only within a certain dynamic range (Egelhaaf and Reichardt 1987). Hence, it needs to be tested by direct electrophysiological analysis of the responses of neurons in the fly's optomotor pathway to optic flow as seen by the animal when walking on the average path of locomotion, whether the system is in a state of optomotor equilibrium on this path. This analysis is currently being done and will be the objective of a subsequent paper (R. Kern et al., unpublished observations).

Although the compensatory optomotor system may well explain that, on average, monocular flies tend to turn towards their open eye, there may be another explanation. It has already been observed long ago that many animal species tend to move on circular paths or, at least turn into one direction, when one of their eyes is occluded (Fraenkel and Gunn 1961). These behavioural responses which are somewhat reminiscent of the behaviour described for flies in the present study have been usually interpreted as a consequence of positive or

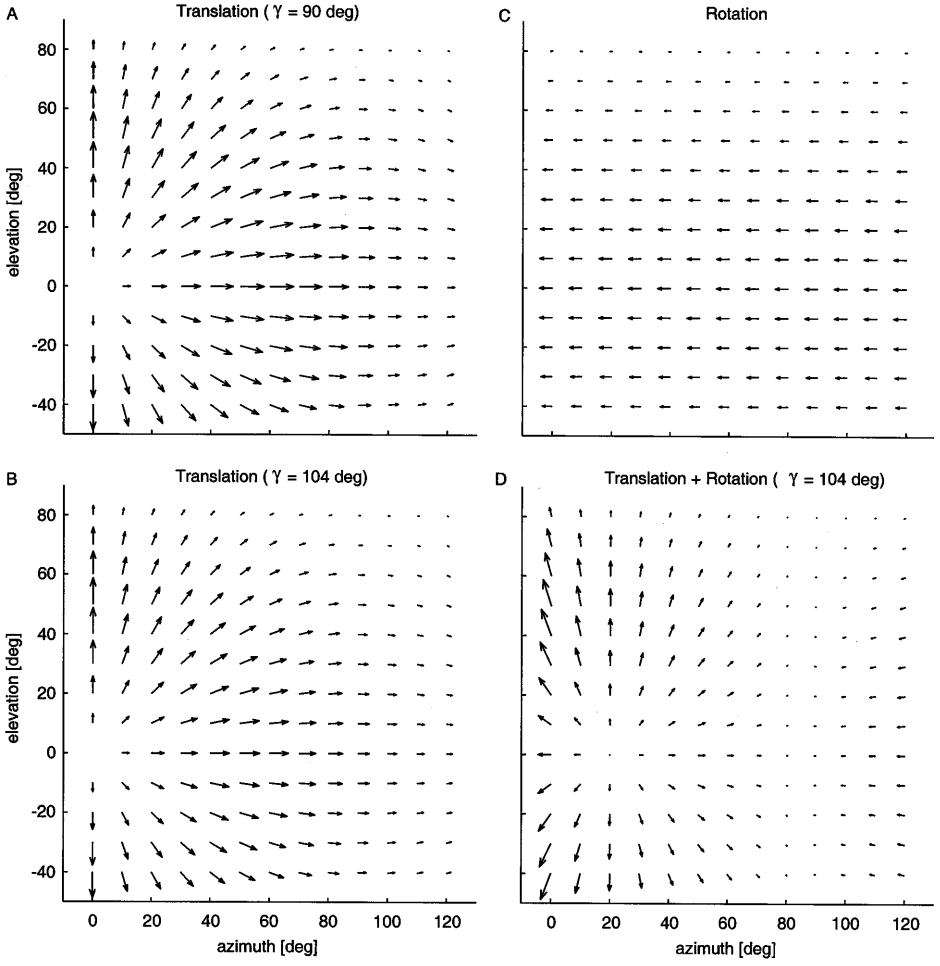

Fig. 5A–D Optic flow calculated for an instant during the average approach of a walking animal towards the arena wall. Calculations were restricted to part of the visual field of the right, i.e. seeing eye, ranging from 0° to 120° in azimuth (0° corresponds to the forward direction) and 80° to −40° in elevation (0° corresponds to the equator of the eye). The local velocity vectors were calculated (for equations see Koenderink and Doorn 1987) for points on the arena wall with an angular spacing of 10°. The local velocity vectors subsequently were transformed into a 2-D Mercator plot. Programmes were written in Matlab (The MathWorks). Distance from the wall always is 39.8 mm. **A,B** Optic flow resulting from pure translation at a velocity of 62.3 mm s^{-1}. In **A** the animal is oriented at $\gamma = 90°$, i.e. the angle subtended by the longitudinal body axis and the tangent onto the arena wall at the intersection point of a line aligned with the longitudinal body axis is perpendicular (see inset Fig. 3C and text). In **B** the calculation was done for $\gamma = 104°$ which corresponds to the median of the average trajectory at a distance to the wall of 39.8 mm. **C** Optic flow resulting from pure rotation to the right about the vertical axis of the animal at 21.4° s^{-1}. **D** Superposition of the translational optic flow shown in **B** and the rotational flow field shown in **C**. **A,B** During pure translation, all velocity vectors point up, down, or to the right, the lengths of the vectors depend on the distance of the corresponding point of the arena to the eye. Since this distance depends on γ, the corresponding vectors are somewhat shorter in **B** than in **A**. **C** During pure rotation to the right, all velocity vectors point to the left, i.e. in the opposite direction of the rotation. **D** Translating and rotating at the same time leads to more complex optic flow with vectors pointing in all directions, their lengths depend on the location of the corresponding points on the arena wall

negative phototactic behaviour. In binocular animals the overall brightness in front of both eyes is balanced, whereas there is an imbalance if one of the eyes is occluded. The animal is then thought to try to reduce this imbalance by trying to turn the eye which is activated less towards the apparent light source in front of the open eye. Since with an occluded eye it is not possible that both eyes are illuminated in the same way, this strategy should lead to continuous turns towards the open eye. Indeed, flies introduced into a homogeneously white arena frequently start to circle (data not shown). This circling about the hindlegs is most prominent in monocular flies which mainly turn in the direction of the open eye. Nevertheless it can be observed also in control animals where circling directions are balanced. However, since the circling is mainly performed on the spot, it strongly differs from the behaviour described in the present paper. Hence, although positive phototactic behaviour can be readily observed in flies (see also Meyer 1978), it is unlikely to play a prominent role in determining the path of locomotion of monocular flies. There is another reason for this conclusion: The angular velocity increases with increasing distance of the animal from the centre of the arena. If the angular velocity were mainly controlled by phototaxis one would expect stronger turns in the centre of the arena than close to the

wall, since there was a slight brightness gradient along the radius of the arena (see Materials and methods). Moreover, the results of the experiments in the flight simulator clearly demonstrate that the turning response depends on the translational velocity of the fly which also speaks against phototaxis as a major determinant of the actual turning velocity when approaching the arena wall.

One might think that the behaviour reported here is – at least to some degree – affected by object fixation. Although this possibility cannot be ruled out, it seems unlikely to us since in the experiments on walking flies we used a random dot pattern and in the experiments in the flight simulator a periodic stripe pattern. Both patterns thus did not contain any prominent object (see also Heisenberg and Wolf 1984).

All this leaves us with the optomotor system as the most decisive determinant of the average trajectories of locomotion as are characteristic of monocular flies.

Flies are free to set a voluntary course of locomotion

As has been pointed out several times in this study, individual trajectories of locomotion of monocular flies only rarely coincide with the average path of locomotion. Rather, they look quite variable and do not differ in an obvious way from those of binocular flies. This just illustrates the trivial fact that animals are not forced by their compensatory reflexes to move straight. In system-analytical terms this means that the setpoint of the compensatory reflexes can be altered arbitrarily depending on other sensory cues in the environment as well as the internal state of the animal. All this is obviously true for normal flies which have access to information provided by both eyes, but also for flies with one eye occluded.

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References

Berg AV v, Brenner E (1994a) Humans combine the optic flow with static depth cues for robust perception of heading. Vision Res 34: 2153–2167

Berg AV v, Brenner E (1994b) Why two eyes are better than one for judgements of heading. Nature (Lond) 371: 700–702

Buddenbrock W v, Moller-Racke I (1952) Beitrag zum Lichtsinn der Fliege *Eristalomya tenax*. Zool Anz 149: 51–61

Collett TS, Nalbach H, Wagner H (1993) Visual stabilization in arthropods. In: Miles FA, Wallman J (eds) Visual motion and its role in the stabilization of gaze. Elsevier, Amsterdam, pp 239–264

Crowell JA, Banks MS, Shenoy KV, Andersen RA (1998) Visual self-motion perception during head turns. Nature Neurosci 1: 732–737

Duffy CJ (1998) MST neurons respond to optic flow and translational movement. J Neurophysiol 80: 1816–1827

Egelhaaf M (1989) Visual afferences to flight steering muscles controlling optomotor response of the fly. J Comp Physiol A 165: 719–730

Egelhaaf M, Borst A (1993a) A look into the cockpit of the fly: visual orientation, algorithms, and identified neurons. J Neurosci 13: 4563–4574

Egelhaaf M, Borst A (1993b) Movement detection in arthropods. In: Wallman J, Miles FA (eds) Visual motion and its role in the stabilization of gaze. Elsevier, Amsterdam, pp 53–77

Egelhaaf M, Reichardt W (1987) Dynamic response properties of movement detectors: theoretical analysis and electrophysiological investigation in the visual system of the fly. Biol Cybern 56: 69–87

Egelhaaf M, Warzecha A-K (1999) Encoding of motion in real time by the fly visual system. Curr Opin Neurobiol 9: 454–460

Fermi G, Reichardt W (1963) Optomotorische Reaktionen der Fliege *Musca domestica*. Abhängigkeit der Reaktion von der Wellenlänge, der Geschwindigkeit, dem Kontrast und der mittleren Leuchtdichte bewegter periodischer Muster. Kybernetik 2: 15–28

Fraenkel GS, Gunn DL (eds) (1961) The orientation of animals. Dover, New York

Frost BJ, Wylie DR, Wang YC (1994) The analysis of motion in the visual system of birds. In: Davies MNO, Green PR (eds) Perception and motor control in birds. Springer, Berlin Heidelberg New York, pp 248–269

Geiger G, Nässel DR (1981) Visual orientation behaviour of flies after selective laser beam ablation of interneurones. Nature (Lond) 293: 398–399

Götz KG (1964) Optomotorische Untersuchung des visuellen Systems einiger Augenmutanten der Fruchtfliege *Drosophila*. Kybernetik 2: 77–92

Götz KG (1968) Flight control in *Drosophila* by visual perception of motion. Kybernetik 4: 199–208

Götz KG (1975) The optomotor equilibrium of the *Drosophila* navigation system. J Comp Physiol 99: 187–210

Hateren JH v, Schilstra C (1999) Blowfly flight and optic flow. II. Head movements during flight. J Exp Biol 202: 1491–1500

Hausen K (1981) Monocular and binocular computation of motion in the lobula plate of the fly. Verh Dtsch Zool Ges 74: 49–70

Hausen K, Egelhaaf M (1989) Neural mechanisms of visual course control in insects. In: Stavenga D, Hardie R (eds) Facets of vision. Springer, Berlin Heidelberg New York, pp 391–424

Hausen K, Wehrhahn C (1983) Microsurgical lesion of horizontal cells changes optomotor yaw responses in the blowfly *Calliphora erythrocephala*. Proc R Soc Lond Ser B 219: 211–216

Hausen K, Wehrhahn C (1990) Neural circuits mediating visual flight in flies. II. Separation of two control systems by microsurgical brain lesions. J Neurosci 10: 351–360

Heide G (1983) Neural mechanisms of flight control in Diptera. In: Nachtigall W (ed) BIONA report. Akademie der Wissenschaften und der Literatur zu Mainz. Fischer, Mainz, pp 35–52

Heisenberg M, Wonneberger R, Wolf R (1978) Optomotor-blind – a *Drosophila* mutant of the lobula plate giant neurons. J Comp Physiol 124: 287–296

Heisenberg M, Wolf R (eds) (1984) Vision in *Drosophila*. Springer, Berlin Heidelberg New York

Heisenberg M, Wolf R (1988) Reafferent control of optomotor yaw torque in *Drosophila melanogaster*. J Comp Physiol A 163: 373–388

Hengstenberg R (1993) Multisensory control in insect oculomotor systems. In: Miles FA, Wallman J (eds) Visual motion and its role in the stabilization of gaze. Elsevier, Amsterdam, pp 285–298

Horstmann W, Warzecha A-K, Egelhaaf M (1999) Synaptic transmission and postsynaptic integration in motion sensitive neurons of the fly (*Calliphora*). In: Elsner N, Eysel U (eds) Göttingen Neurobiology Report 1999. Thieme, Stuttgart, p 444

Ibbotson MR, Goodman LJ (1990) Response characteristics of four wide-field motion-sensitive descending interneurons in *Apis mellifera*. J Exp Biol 148: 255–279

Kern R, Nalbach H, Varju D (1993) Interactions of local movement detectors enhance the detection of rotation. Optokinetic experiments with the rock crab, *Pachygrapsus marmoratus*. Vis Neurosci 10: 643–652

Kern R (1998) Visual position stabilization in the hummingbird hawk moth, *Macroglossum stellatarum* L.: II. Electrophysiological analysis of neurons sensitive to wide-field image motion. J Comp Physiol A 182: 239–249

Kern R, Varjú D (1998) Visual position stabilization in the hummingbird hawk moth, *Macroglossum stellatarum* L.: I. Behavioural analysis. J Comp Physiol A 182: 225–237

Koenderink JJ, Doorn AJ v (1987) Facts on optic flow. Biol Cybern 56: 247–254

Krapp HG, Hengstenberg B, Hengstenberg R (1998) Dendritic structure and receptive-field organization of optic flow processing interneurons in the fly. J Neurophysiol 79: 1902–1917

Meyer HW (1978) Phototaxis in the walking male and female fly (*Calliphora erythrocephala* Meig). I. The spontaneous phototactic reaction. J Comp Physiol 123: 307–314

Nalbach G (1993) The halteres of the blowfly *Calliphora:* I. Kinematics and dynamics. J Comp Physiol A 173: 293–300

Nalbach G, Hengstenberg R (1994) The halteres of the blowfly *Calliphora*. II. Three-dimensional organization of compensatory reactions to real and simulated rotations. J Comp Physiol A 175: 695–708

Pringle JWS (1948) The gyroscopic mechanism of the halteres of Diptera. Philos Trans R Soc Lond B 233: 347–385

Reichardt W, Poggio T (1976) Visual control of orientation behaviour in the fly. Part I. A quantitative analysis. Q Rev Biophys 9: 311–375

Sachs L (ed) (1984) Applied Statistics. Springer, Berlin Heidelberg New York

Schaeffer AA (1928) Spiral movement in man. J Morphol Physiol 45: 293–398

Simpson JI, Leonard CS, Soodak RE (1988) The accessory optic system of rabbit. II. Spatial organization of direction selectivity. J Neurophysiol 60: 2055–2072

Srinivasan MV, Lehrer M, Kirchner WH, Zhang SW (1991) Range perception through apparent image speed in freely flying honeybees. Vis Neurosci 6: 519–535

Strauss R (1991) Das Laufverhalten von *Drosophila melanogaster* und seine Beeinflussung durch genetisch gesetzte Läsionen im Zentralkomplex. Dissertation, Universität Würzburg

Strauss R, Heisenberg M (1990) Gaze stabilizing head movements compensate for walk-induced body oscillations in the fly *Drosophila melanogaster*. In: Elsner N, Roth G (eds) Brain-perception-cognition. Thieme, Stuttgart, pp 63

Tracey D (1975) Head movements mediated by halteres in the fly, *Musca domestica*. Experientia 31: 44–45

Warren WHJ, Blackwell AW, Kurtz KJ, Hatsopoulos NG, Kalish MJ (1991) On the sufficiency of the velocity field for perception of heading. Biol Cybern 65: 311–320

Warren WHJ, Hannon DJ (1990) Eye movements and optical flow. J Opt Soc Am A 7: 160–169

Wehner R (1981) Spatial vision in arthropods. In: Autrum H (ed) Handbook of sensory physiology, vol VII/6C. Comparative physiology and evolution of vision in invertebrates. Springer, Berlin Heidelberg New York, pp 287–616

Wendler G, Scharstein H (1986) The orientation of grain weevils (*Sitophilus granarius*): influence of spontaneous turning tendencies and or gravitational stimuli. J Comp Physiol A 159: 377–389