Thorax movements during blowfly walking

Summary

Visual information processing depends to a large extent on the own movements of an animal. To investigate these movements in freely walking blowflies, we measured their orientation and position with high spatial and temporal accuracy by attaching tiny sensor coils to head and thorax. Experiments were performed on Calliphora vicina, Lucilia cuprina and Lucilia caesar. During walking, the thorax orientation of the flies is often different from the direction of walking; differences of 45 degrees are common, and there are even short traces of backward walking. However, the head and thorax orientations are aligned most of the time, regardless of the misalignment of the thorax orientation with the walking direction. The orientation of the thorax changes abruptly with a frequency typically between 5 and 10 Hz; between these saccades the orientation is relatively constant. Also the changes in position are saccadic, and the two types of saccades occur at roughly the same time. On average, though, the orientation saccade leads the position saccade. The walking velocity can reach 15 - 20 cm/s, but 90% of the time it is less than approximately 6 cm/s. The yaw velocity can reach 1500 - 2000 degrees/s, but 90% of the time it is under 200 degrees/s. Several turning manoeuvres similar to those previously described in Drosophila melanogaster are observed; the flies turn either gradually by walking on a ‘curved path’ or they turn abruptly ‘on the spot’.
Introduction

Visual systems are most likely optimised, through evolution, for the processing of natural visual input. Since this input is to a large extent determined by the own movements of the animal, it is important to study these movements during normal behaviour. In flies, movements of thorax and head were studied in detail during flight (Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999). Here we extend these measurements to walking flies. Vision during walking may be important for flies, because they spend much of their time walking, for example while searching for food in their proximity (Dethier, 1976). Furthermore, walking in flies is much studied, because it is easier to record than flight. For example, Kern et al. (2001) use recorded walks of Lucilia to present reconstructed optic flow fields as stimuli to study movement sensitive neurons in the fly brain.

Most existing methods of recording insect movements are based on video techniques (Sandström et al., 1996; Fry et al., 2000; Tammero and Dickinson, 2002). They usually have a limited temporal resolution, limited spatial resolution for small subjects in large spaces, and they cannot readily measure simultaneously all six degrees of freedom of the head. Apart from the video techniques, there is one method employing search coils (Schilstra and van Hateren, 1998a), which has been previously applied in the study of flying insects. It allows the simultaneous reconstruction of the head and thorax orientation with high spatial and temporal resolution, permitting a complete reconstruction of the spatio-temporal visual input. A disadvantage of the technique compared with video recording is the attachment of coils and wires to the fly. For the measurements presented here this is not a problem (see Materials and Methods). We use the method to study the normal walking behaviour in several species of blowflies. In this chapter we concentrate on thorax movements, and how turns are made during walking. In the next chapter, we will concentrate on head movements and some of their consequences for the visual system.
Materials and methods

Flies and coils

Experiments were performed on female blowflies; the results are based on 3 specimens of *Calliphora vicina*, 2 of *Lucilia cuprina* and 2 of *Lucilia caesar*. The position and orientation of the head and thorax of the flies walking in a cage was recorded by attaching small coil triplets to the flies. These coil triplets pick up externally applied magnetic fields and from these signals position and orientation can be reconstructed (Schilstra and van Hateren, 1998b).

Attaching coils to the head and thorax may potentially interfere with normal movements. Therefore, we took into account the control experiments of van Hateren and Schilstra (1999) for determining the maximum acceptable coil sizes. They found that, for *Calliphora vicina*, coils of 40 windings (2 mm diameter, triplet weight 0.8 mg) attached to the head, and 80 windings (2 mm, 1.6 mg) attached to the thorax, had a negligible effect on the head and thorax motion during flying. We used coils of the same dimensions in the present study on walking *Calliphora*. Because accelerations, both of translation and of rotation, are considerably lower during walking than during flying, we expect that the effect of the coils is also negligible in this case. *Lucilia cuprina* is smaller than *Calliphora vicina*, and the body mass of the former (approximately 45 mg for the flies used in this study) is almost three times lower than the body mass of the latter (approximately 130 mg for the flies used in this study). The head weight is approximately the same for both species. An additional problem with *Lucilia* is that the small size of the fly does not allow coils of 2 mm to be attached to both head and thorax: they would touch each other. We therefore used coils of 1 mm diameter (40 windings, triplet weight 0.2 mg) for the head of *Lucilia*, and coils of 2 mm diameter (40 windings, 0.8 mg) for the thorax. We expect that the effect of these coils (proportionally smaller than those used for *Calliphora*) on normal movements is negligible.
Preparation, experiment and data processing

Preparations for attaching the coils were similar to those used by van Hateren and Schilstra (1999). The main difference is that we clipped the tips of the flies’ wings. The flies were immobilised in a padded clamp, allowing access to the dorsal part. Subsequently, the hairs that impeded the attachment of coils or wires were removed and the sensor coils were glued with cyanoacrylate on top of the head and on the thorax, midway between the wings. The coils were oriented in close alignment with the symmetry axes of the fly. The cable corresponding to the head coil made a loop to allow free head movements, and was connected to the thorax, both cables were connected to the last segment of the abdomen, and the end of the cable was connected to the recording equipment. The flies were fed with water and sugar, and then released in the arena. The walking arena consisted of a cylinder (225 mm diameter and 80 mm height), with the walls covered with a random pattern of smoothed black and white squares (50% probability of occurrence), each subtending 10 degrees when viewed from the centre of the cage. The bottom was covered with a texture of light grey squares on a dark grey background. Every two to three hours the flies were fed again, and the cables were checked for loops, which would have hindered free walking.

The technique for measuring the position and orientation of the head and thorax is described by Schilstra and van Hateren (1998a) and will be briefly summarised here. Three orthogonal pairs of coils surrounding the measurement space were driven at three different frequencies (50, 68, and 86 kHz) and generated one gradient and two homogenous magnetic fields. Two sensor coil triplets attached to the head and thorax of the fly consisted each of three orthogonal coils. The coils picked up voltages induced by the magnetic fields, and were each connected to three lock-in amplifiers (eighteen in total). The lock-in amplifiers connected to a particular coil were tuned to the frequencies of the driving fields. The output of the lock-in amplifiers thus provided nine values per coil triplet, sufficient for reconstructing the six degrees of freedom (three positional and three angular coordinates) per coil triplet. The outputs of these amplifiers were digitised and stored for offline analysis. The accuracy of the method with the coils used for Calliphora is approximately 1 mm, 0.3 deg, and 1 ms. The coils used for Lucilia had fewer windings and a smaller diameter for the head triplet, and thus picked up less magnetic flux. The decreased signal strength and
decreased signal-to-noise ratio reduced the accuracy to an estimated 2 mm and 0.5 degrees. We will therefore concentrate in this chapter on the results obtained on *Calliphora*, and only discuss the results for *Lucilia* where appropriate.

The reconstructed data was low-pass filtered with a two-sided exponential filter, with time constants of 15 ms for position, 5 ms for head orientation, and 3 ms for thorax orientation. Walking and turning speeds were computed by differentiating position and yaw, respectively. For the analysis we only used data that corresponded to walking (see Results).

Slight variations in the alignment of the coil triplets from fly to fly were corrected by centring the distributions of head and thorax roll and pitch, adjusting the thorax yaw such that a symmetrical distribution of transversal horizontal speed of the thorax resulted, and centring the distribution of the head yaw in the thorax coordinate system. All necessary corrections were smaller than 10 degrees.

**Angular coordinates**

The spatial orientation of an object is described by three angles corresponding to an ordered sequence of rotations about three orthogonal axes, and starting from a reference orientation. We use here the Fick convention, which is a passive reference system - meaning that the reference system, including the object, rotates about its own axes in a fixed sequence (Haslwanter, 1995): a rotation about the vertical axis of the object, followed by a rotation about its transverse axis, and then by a rotation about its longitudinal axis. Each rotation is thus performed about an axis resulting from the previous rotation. In the present chapter all orientational angles refer to yaw, *i.e.*, rotation about the vertical axis. The yaw velocity is calculated in the coordinate system attached to the thorax, instead of the laboratory coordinate system.
Figure 3.1. Typical walking path of a blowfly during 13 seconds. The arrows marked x and y show the scale (1 cm). The curled line denotes the walking trace, short black lines (drawn every 50 ms) the yaw orientation of the thorax (loose ends of lines point into head direction of the thorax), and grey lines the yaw orientation of the head. The numbers indicate the time elapsed (in seconds) from the start of walking. Open circles mark maxima of the yaw velocity, small black dots and large grey dots mark maxima and minima of the walking velocity, respectively.
Results

Figure 3.1 shows an example of 13 seconds of walking by *Calliphora vicina*, on the horizontal plane. The black curled line represents the trace of walking, and connects the successive positions of the thorax centre. The short black lines indicate the position and orientation of the thorax every 50 ms and the grey lines depict the corresponding orientation of the head. These lines start from the position of the thorax centre and point frontward, in alignment with the head (grey) and thorax (black). The numbers in the figure mark the time elapsed, in seconds, from the start of walking. The circles and dots will be explained below. As can be seen, the walking path contains both smooth curves and abrupt changes of direction of movement.

The most striking feature of walking observed from this graph is that the orientation of the thorax is often misaligned with the direction of walking. For example, at seconds 1.7 (arrow a) and 6.7 (arrow b) - just after the right angle turns - the difference is almost 90 degrees. Careful examination of the figure reveals that there are even short sections where the fly is walking backwards, just after the sharp (> 90 degrees) turns (examples at 3.9 s, arrow c; 4.3 s, arrow d; 5.4 s, arrow e). The direction of walking may change very abruptly, but the orientation of the thorax generally changes during 1-2 cm of walking; this change contains fine structure which will be discussed below. As a consequence, the thorax orientation is, either before or after a sharp turn, often significantly different from the direction of walking. We found this difference between the orientation and direction of walking in all flies from the three species we investigated (*Calliphora vicina, Lucilia cuprina* and *Lucilia caesar*).

Another interesting feature that can be seen in Figure 3.1 is that the head is roughly aligned with the thorax. Thus the fly does not orient its head towards the direction of walking in order to offset the thorax misalignment, although it is capable to turn the head by up to 20 degrees (Hengstenberg et al., 1986). In Figure 3.2 we have plotted a histogram of the differences between the head and the thorax yaw angle for the data shown in Figure 3.1. The distribution has a standard deviation of 2.6 degrees, and shows that the head and the thorax are closely aligned on average. The shoulder at the right side of the distribution is a consequence of the limited amount of data,
and it vanishes when using more data (graph not shown), where the distribution becomes close to a Gaussian. Similar histograms were obtained for all flies from all species studied, with standard deviations ranging between 6 and 11 degrees.

Saccades

In order to investigate the relationship between thorax orientation and the direction of walking, we have plotted the yaw angle (Figure 3.3A) and x and y coordinates of the thorax (Figure 3.3B) during a short interval (seconds 3-6 from Figure 3.1). It is apparent from the graphs that yaw angle and position coordinates are composed of intervals of almost constant orientation or position, interrupted by fast saccadic turns or position changes. We will designate as “orientation saccades” the fast saccadic turns and as “position saccades” the fast position changes.

Figure 3.3C depicts the yaw velocity (corresponding to figure 3.3A), and gives an idea about the frequency and angular velocity of thorax orientation.
Figure 3.3. Orientation, position, and velocities during 3 seconds of walking. (A) Yaw angle of the thorax. (B) x and y position of the thorax. Note that orientation and position change stepwise (through saccades). (C) Yaw velocity. (D) Walking velocity (thick line) and absolute yaw velocity (thin line; vertical scale not shown, trace is the absolute value of Fig. 3C). Peaks of the walking velocity have corresponding peaks in the yaw velocity.
saccades. They are brief, in the order of 1000 degrees/s, and succeed at a rate of 5 – 10 Hz. Between saccades, the thorax orientation is almost constant. The thick line in Figure 3.3D shows the total walking velocity (length of the velocity vector). Peak walking velocities correspond to position saccades. Unlike the intervals between orientation saccades where the yaw angular velocity returns to zero, in between position saccades there are troughs of lower walking velocity. These troughs do not become zero, indicating that the fly does not stop walking. Only during complete breaks both angular velocity and walking velocity become zero (e.g. at 5.5 s in Figure 3.3). The thin line in Figure 3.3D, superimposed on the walking velocity, represents the absolute yaw velocity (from Figure 3.3C), and is shown (without vertical scale) on the same graph with the walking velocity to facilitate the comparison of the timing of position and orientation saccades. It is apparent that almost every peak in the walking velocity corresponds to a peak of the absolute yaw velocity, thus the saccades of thorax orientation and position occur at roughly the same time. However, there seems to be no correlation between the sizes of the corresponding saccades. In order to observe the temporal order of the two types of saccades in Figure 3.1, the peaks of absolute yaw velocity are marked there with open circles. Small black dots and large grey dots indicate peaks and troughs of the walking velocity, respectively. This coding facilitates observing a sequence which repeats itself in most of the walking: minimum walking velocity – peak yaw velocity – peak walking velocity (large grey dot – open circle – small black dot). We observed this characteristic sequence during walking in all flies of the three species studied. The time intervals between orientation saccades and the corresponding position saccades are plotted in a histogram in Figure 3.4, using the data shown in Figure 3.1. Corresponding pairs were determined by finding, between every two neighbouring troughs of the walking velocity, the peak yaw velocity and the peak walking velocity. The histogram shows the difference between their times of occurrence. A positive time interval signifies that a position saccade follows an orientation saccade. On average, the peak walking velocity is attained 9.3 ms (± 17.7 ms, SD) after the peak yaw velocity. Thus there is a slight ordering of the orientation and position saccades, which can also be observed in Figures 3.1 and 3.3D.

For vision, the distributions of the yaw velocity and the walking velocity of the thorax are important, because they determine, through corresponding
head movements, the rotational and translational components of the optic flow. We collected all yaw velocities during walking in a histogram with a bin size of 100 degrees/s, and then normalized it to get the probability density function (p.d.f.). In order to discriminate between walking and breaks, we applied a double-sided exponential filter to the walking velocity, with a time constant large enough to decrease the amplitude of the 5 – 10 Hz peak-trough oscillations. Subsequently, we separated the data in walking or break by using a velocity criterion. We found that a good separation was obtained with a time constant of the filter of 150 ms, and a criterion velocity of 0.8 cm/s. The line connecting filled circles in Figure 3.5A represents the p.d.f. of yaw velocity for the data set shown in Figure 3.1. We also computed the p.d.f. of yaw velocity during walking for the three Calliphora (each with 20 – 25 minutes of walking). The thick line in Figure 3.5A represents the average p.d.f. of the three distributions, with the error bars corresponding to the standard deviation (computed in logarithmic coordinates). The resulting p.d.f. is slightly less steep than an exponential distribution. The distribution of yaw velocity for the 13 s data set follows the average distribution reasonably well. The differences in the low probability range (10-2-10-4) are most

Figure 3.4. Histogram of time intervals between yaw velocity peaks and corresponding peaks of the walking velocity for the data of Figure 3.1; the average separation is 9.3 ± 17.7 ms. A positive interval means that the peak of the walking velocity occurred after the peak of the yaw velocity.
likely due to the small number of samples. Although there are angular velocities reaching 1500 – 2000 degrees/s, most of the time the angular velocity

Figure 3.5. (A) Probability density of thorax yaw velocity for the data of Figure 3.1 (filled circles connected by thin lines) and for 4497 s of walking from three Calliphora (thick line with error bars). (B) Probability density of walking velocity for the data of Figure 3.1 (filled circles connected by thin lines) and for 4497 s from three Calliphora (thick line with error bars).
is much lower, corresponding mostly to areas “between saccades” when the yaw angle is relatively constant. For *Calliphora* we found that 90% of the angular velocities are lower than 200 degrees/s (3 flies, 4497 s of walking), for *Lucilia cuprina* 200 degrees/s (2 flies, 1713 s), and for *Lucilia caesar* 230 degrees/s (2 flies, 472 s). The fact that the histograms of Figure 3.5A are smooth is consistent with the abundance of saccades: saccades occur in a continuous range of sizes, and each saccade covers a continuous range of yaw velocities.

Figure 3.5B shows the distributions of walking velocities for the 13 s dataset and the large 4497 s *Calliphora* dataset also used for Figure 3.5A. Again, the 13 s distribution follows the average distribution well, and differences appear only at low probabilities. Except for the first bin which corresponds to walking velocities between 0 and 1 cm/s, the distribution closely follows an exponential distribution. Although there are instantaneous walking velocities of 15 – 20 cm/s, most of the time they are much lower: 90% of the walking velocities are lower than 6.3 cm/s (*Calliphora*), 6.2 cm/s (*Lucilia cuprina*), and 5.3 cm/s (*Lucilia caesar*).

**Turning manoeuvres**

Figure 3.6 shows typical manoeuvres performed by flies during walking; these manoeuvres are common to all flies of each species we have studied, and at least one of the manoeuvres appears at each change in the direction of walking. Similar manoeuvres were described for *Drosophila melanogaster* by Wannek and Strauss (1996), and we use their terminology, designating by “turns on the spot” the abrupt changes of orientation over a very short distance and by “curved paths” the gradual changes. We further distinguish between “3-phase turns”, having a brief phase of backward movement, and the similar “2-phase turns”, which lack the backward phase.

Figure 3.6A depicts a typical walk on a curved path, during which the fly performed a turn of approximately 40 degrees (detail from Figure 3.1 rotated by 90 degrees, approximately seconds 9 – 10). On curved paths, the thorax orientation is not very different from the direction of movement. On such paths, the direction of walking changes gradually, while the orientation changes abruptly and repeatedly in a series of saccades. Hence the orienta-
Figure 3.6. Typical manoeuvres performed by flies during walking. Thorax orientations (short lines) are shown every 20 ms, open circles denote maxima of the yaw velocity, small black dots indicate the maxima of the walking velocity, and large grey dots mark minima of the walking velocity. The numbers denote time elapsed (in ms) from the start of walking. (A) Turn on a curved path. (B) 2-phase turn. (C) 3-phase turn.
tion and direction do not follow each other exactly, yet they are not too different; normally they differ less than 45 degrees. The orientation may be ahead or behind the direction of walking.

Figure 3.6B shows a 2-phase turn on the spot with a total turn of approximately 65 degrees. The first phase, consisting of an abrupt change of direction, occurs at approximately 490 ms. The second phase consists of progression in the new direction; at the onset of this phase, the fly moves in the new direction, yet it is oriented almost in the old direction. The thorax orientation changes towards the direction of movement in a series of saccades (three shown). Often during the second phase (while the direction and orientation are still different), a new 2-phase turn to the opposite direction is initiated; in this case, after the first phase of the second turn, the direction and orientation are almost aligned (example in Figure 3.1, arrow f, 6.8 – 6.9 s). Even series of successive 2-phase turns oriented in opposite directions are possible, with little changes of orientation but abrupt changes in direction; the trace has a zigzag appearance, while the orientation changes much less (Figure 3.1, 0 – 1 s, 5 – 7 s).

Figure 3.6C shows a 3-phase turn on the spot with a total turn of approximately 95 degrees. The first phase starts at 440 ms by a backward motion combined with rotation (two saccades of 20 and 30 degrees). At the onset of this phase the direction of movement changes abruptly by approximately 110 degrees. During the second phase (around 600 ms), the direction of movement changes smoothly by approximately 100 degrees, while the orientation does not change. In the last phase the fly is already progressing towards the new direction. At the beginning of this last phase, the walking direction and the orientation are different by almost 90 degrees and they converge until they become aligned.

In turns, the direction of walking can be very different from the orientation, with the thorax orientation leading or lagging the direction of walking. The angle between thorax orientation and walking direction can exceed 90 degrees for brief periods following turns on the spot, and on curved paths it can reach 45 degrees. Also while walking on a curved path and throughout all phases of turning on the spot, the orientation of the thorax changes in a series of saccades; between saccades, the orientation is relatively steady. All turning manoeuvres occur in each fly from the three species studied. Curved
paths are generally used for small changes in walking direction. For larger
changes (up to approximately 90 degrees), performed in a short time, 2-
phase turns are commonly used; for even larger changes, one typically finds
3-phase turns.

Discussion

During walking, the thorax orientation of the flies Calliphora vicina, Lucilia
cuprina and Lucilia caesar is often misaligned with the walking direction.
This difference reaches 45 degrees on curved paths and up to 90 degrees
while turning on the spot, in agreement with similar results reported for
Drosophila melanogaster by Wannek and Strauss (1996). The head and tho-
rax are roughly aligned. Although the head is capable of turning by 20
degrees with respect to the thorax (Hengstenberg et al., 1986), it does not
try to compensate for the thorax orientation offset. Thus, whenever a fly
walks sideways, which it frequently does, the optic flow is not symmetrical,
and this should have important consequences for vision. Regardless of the
manoeuvres involved, the changes in orientation are always saccadic with a
frequency of approximately 5 – 10 saccades/s. Similar results are obtained
for flying Calliphora vicina (Schilstra and van Hateren, 1999) and Lucilia
(unpublished observations). Between saccades, the thorax orientation is rel-
atively stable. Several systems could be involved in this stabilisation
(Hengstenberg, 1993): the visual feedback and the halteres (Sandeman and
Markl, 1984). Unlike the orientation changes, the walking direction may
change gradually, and it may also change abruptly by large amounts of even
180 degrees. The position saccades and orientation saccades usually coin-
cide, with a slight ordering: on average, the orientation saccades occur
slightly earlier than the position saccades.

Two different types of movements of walking Calliphora erythrocephala have
been previously reported (Horn and Mittag, 1980): “type I” movements
have relatively large and slow orientation changes (28 - 65 degrees, 0.4 - 1.3
Hz) and are not present in all flies, while “type II” movements are small and
frequent (5.9 - 8.2 degrees, 7.3 - 10.6 Hz), and are always present. In view
of the results in the present chapter, it appears likely that type II move-
ments are actually saccadic orientation changes. The type I movements pos-
possibly correspond to the large, corner-like turns in Figure 3.1, which in fact consist of a compound series of saccades.

In the present visual surround (a background of random dots, without other objects), the blowflies display a searching behaviour, stopping and changing direction frequently. The peak momentary walking velocities we found were 15 – 20 cm/s, but 90% of the time the walking velocity was less than approximately 6 cm/s. Higher average walking velocities could probably be attained with a Buridan’s paradigm (Götz, 1980; Bülthoff et al., 1982). In this paradigm, flies run continuously on nearly straight lines between two opposing and inaccessible black objects on a white background. The walking velocity of Calliphora is obviously lower than the flying velocity in a wind tunnel: 2-3 m/s (Nachtigall and Roth, 1983), or in a restricted volume: up to 1.2 m/s (Schilstra and van Hateren, 1999).

Several patterns of turning, namely turning on curved paths or on the spot (2-phase and 3-phase turns), often occur; similar turning patterns were observed in Drosophila melanogaster by Strauss and Heisenberg (1990) and Wannek and Strauss (1996). Throughout curved paths, the direction of walking changes gradually while the orientation changes in a sequence of small and abrupt saccades. The angle between the direction of walking and the orientation is usually lower than 45 degrees. More drastic turns are performed by suddenly changing the direction of walking (“turning on the spot”). During a 2-phase turn, one abrupt change of up to 90 degrees occurs. During a 3-phase turn the first abrupt turn may reach up to 180 degrees and is followed by backward walking. Another fast direction change (abrupt or more gradual) eventually results in forward walking. We found that while performing these manoeuvres flies carry out saccadic changes in thorax orientation and never gradual ones. In general, we hardly observed any differences in the walking behaviour between the three fly species studied. This finding is consistent with the fact that they are closely related to each other.

References

Chapter 3


