Tri-axial, real-time logging of fly head movements

Gert Stange 1, Roland Hengstenberg
Max-Planck-Institut, für biologische Kybernetik, Spemannstrasse 38, D-72076 Tübingen, Germany

Received 23 January 1995; revised 13 August 1995; accepted 3 October 1995

Abstract

We present a method to record and simultaneously display the three rotatory components of arbitrary head turns of an insect flying stationarily in a wind tunnel or walking on a treadmill. An elongated marker, placed on the fly's forehead, is video-recorded from ahead under deep red stroboscopic illumination, invisible to the insect. A fast on-board image processor of a PC video-adapter (True Vision, AT-Vista), programmed in its native code, extracts position and orientation of the marker in the video-image. The host PC transforms these data into calibrated head angles and displays stimulus and response components after 40 ms processing time at a rate of 50 frames per second. Head turns are measured relative to the fly's trunk even when the fly is rotated around its body axis provided that it is aligned with the video-axis. Technical tests, as well as recordings from live flies responding to various stimuli, illustrate the performance and accuracy of the procedure. This minimally invasive method of motion recording should be easily adaptable to other insects and to similar movements of small parts.

Keywords: Video tracking; Motion recording; Image analysis; Gaze control; Head movement; Insect vision; (Fly); Calliphora

1. Introduction

For an organism capable of vision, it is important to be able to rapidly and precisely control the position of the eyes (Laurentius, 1599, cited in Carpenter, 1988), in order to shift gaze and, during egomotion, to stabilize the image of the surroundings. The sensory and motor systems that control eye orientation in vertebrates constitute a biological servo system of considerable theoretical interest which has been subject to numerous investigations. To obtain experimental data, a wide range of position logging methods has been employed (e.g., Crane and Steele, 1985; Ferman et al., 1987; Hartmann and Klinke, 1976; Koch, 1977, 1983; Marelli and Hsiao, 1976; Miall and Hereward, 1988; Ott and Eckmiller, 1989; Robinson, 1963; Sandeman, 1968; Yarbus, 1967; Young and Sheena, 1975; Zarnack, 1978)

In insects, the eyes are fixed relative to the head and actually take up the majority of its volume. Consequently,
manual or semiautomated system, where an operator has to inspect every individual frame, rapidly becomes unendurable if many or long sequences are to be analyzed.

Therefore, we developed an automated method to record head turns of the blowfly *Calliphora erythrocephala* about all 3 body axes simultaneously, and to record and display stimuli and responses in real time. Since head movements in flies are also influenced by the mechanical sensation of body turns through the halteres (Hengstenberg, 1988), the measuring procedure should also allow rotations of the fly about one of its body axes. Taking the fly's visual system into account, we aimed at an angular resolution of 1-2°, a signal-to-noise ratio of at least 5:1, less than 5% deviation from linearity over the ranges of head turning, and as little crosstalk as possible between signals from the different axes. The method illustrated here can be easily adapted for other experimental problems with similar constraints.

2. Materials and methods

2.1. Mechanical layout, position control

The mechanical components of an existing semiautomatic system were used without alterations (Fig. 1). A wire bracket is attached to the thorax of a fly and mounted coaxially to the shaft of a servo motor (FM); it is of crucial importance that the axis of the head (see below, Fig. 3) is accurately aligned with the axis of the apparatus. The fly can be rotated around the roll axis, using a closed-loop analog position servo system in which position is encoded as a voltage by a ring potentiometer (FP). The voltage is also A-D-converted and sampled by the laboratory Computer. In addition, the computer outputs an analog signal which is used as the servo control voltage. Thus, the fly can be positioned, under computer control, at any angle or be rotated at any velocity up to 2500°/s. Body rotations act upon one of the fly's flight equilibrium organs, the halteres.

The eyes form another part of the fly's flight stability control system. To provide visual stimulation, a pattern cylinder is mounted concentrically around the fly and position-controlled in the same way by a second servo system with the components PM, PP. The pattern cylinder simultaneously serves as a wind tunnel; an air flow of 2 m/s (arrows) is generated by a fan on the downwind side of the motors. The apparatus can be mounted with the axis oriented vertically, excluding any effect of gravitation an the orientation of the fly, even though gravitation sense organs are not known.

2.2. Illumination, camera

In order to make visual stimulation of the fly independent of the illumination needed for the video camera, two light sources are provided. One is optimized to match the sensitivity of the fly's visual system, the other optimized to match the sensitivity of the video camera. Simultaneously, care is taken to exclude crosstalk.

The stimulus light source consists of 3 daylight circular fluorescent tubes (Philips TL 40 W), driven by a high-frequency power supply (Philips BRC 410) to ensure freedom from flicker. It illuminates a translucent diffuser cylinder, providing uniform illumination of the outside of the pattern cylinder, which is also translucent. The spectrum of the light source is adequate to excite the photoreceptors mediating visual motion perception (Hardie, 1985; Heisenberg and Buchner, 1977). The low emission at short wavelengths, however, is inadequate for eliciting. UV-dependent responses (Fig. 2a,c).
Fly vision cuts off at wavelengths above about 570 nm; therefore, using an infrared light source and camera minimizes optical crosstalk from the measuring system to the visual system. The video camera (Kappa HR600S) is equipped with an electronic shutter. Illumination optimized to the spectral sensitivity of the camera (Fig. 2b,d) is provided by a xenon stroboscope (Brüel & Kjaer, mod. 4913, 4915) which illuminates the fly via an infrared cut-on filter (Schott RG 670) and a ring light guide (VOLP1). The spectral separation of the stimulus illumination (Fig. 2a) and the measuring flashes (Fig. 2b), combined with the restriction of the spectral sensitivity of the camera to long wavelengths (Figs. 1 and 2d) ensures that the intensity of the unwanted stimulus light an the video target is about 1500 times less than that of the measuring flashes. Secondly, the flashes (3 μs half-time) are synchronized with the camera shutter. Their full brightness is captured during the shutter open time of 100 μs. The stimulus illumination, on the other hand, is continuously on. Since the camera shutter is closed for 19.9 ms out of 20.0 ms frame duration, only 1/200 of the total stimulation light reaches the video target.

This lighting arrangement fulfills several conflicting requirements at the same time. (1) The fly can see the stimulus pattern continuously without crosstalk from the measuring flashes (Fig. 2a-c). (2) As a result of the combination of the spectral and temporal separation factors, the crosstalk from the stimulus illumination to the video camera is about 300,000 times less than the brightness of the measuring flashes. This is much below the grey level resolution (1 in 256) of the video adapter; hence the head position measurements are not at all disturbed by the stimulus pattern illumination. (3) The very short exposure time of image capture eliminates any motion blur that might be caused by rapid head movements of the fly or by imposed body rotations (Figs. 7, 9 and 10).

2.3. What the camera sees

Fig. 6a shows an image of a fly’s head, taken under the described illumination conditions. The image is adequately structured to allow, in principle, totally non-invasive determination of position and movement by existing image analysis algorithms (e.g., Srinivasan, 1994) but, to obtain real time processing speed, the demands a computing power are not within the reach of the available equipment.

Therefore, it was decided to use an approach that minimizes the computational task, albeit at the expense of being slightly invasive: in the majority of experiments, a white oblong marker of zinc white was painted with a fine water-colour brush (size 000) onto the forehead of the fly (Fig. 3), which is held within a plastic foil collar under a dissecting microscope. The use of reflecting foil (3M Scotch Lite High Gain) was also tested. This material has a very high reflectivity with coaxial illumination. But with oblique illumination as provided by the ring light guide, its reflectivity is not better than that of zinc white. Since the latter is easier to apply, and the contrast suffices for discrimination, we prefer zinc white for the current investigations. The positioning of the marker is dictated by the fact that it must not interfere with sense organs including eyes, ocelli and antennae. The fly’s neck joint allows the head to be turned in all directions but only minute translations (Strausfeld et al., 1987). Video observations from the side, at high magnification, showed that the distance between the prothorax and the base of the antennae (Fig. 3a) varies less than 5% in different locomotor states (Hengstenberg, unpublished). Hence the marker on the fly’s forehead moves essentially on the surface of a sphere.

The fortunate anisometry of head turning ranges (yaw and pitch: ±20°; roll: ±90°) allows to measure the 3 head angles simultaneously in a fronto-parallel image of the fly’s face: yaw turns cause horizontal shifts of the marker, pitch turns cause vertical shifts, and roll turns cause rotations of the marker in the image plane (Fig. 3). For accurate roll measurements, the marker must be distinctly elongated; the 180° ambiguity of the marker orientation is resolved by its eccentric position relative to the head axis.

Determination of the yaw and pitch angles requires a trigonometric correction for the off-axis position of the marker and scaling for the size of the head (Fig. 3a). The scaling factor was determined from sagittal sections of the fly’s head by the ratio of distances between the occipital condyles of the neck joint and the marker, and the distance between the ocelli and the edge of the labrum (Figs. 3 and 6h), assuming that flies of slightly different size maintain this proportion.
2.4. Algorithm

Given the above, a skeleton solution to the task of determining position in real time, at video rate, is as follows:

(1) capture frame of video in the video memory of a frame grabber;
(2) threshold image such that only the pixels forming part of the marker are set;
(3) treat the set pixels as an oblong geometric figure or cluster of \( N \) points. For such a figure (K.G. Götz, pers. comm.) the coordinates of the centre of gravity are:

\[
\begin{align*}
x_c &= \frac{\sum x_i}{N} \\
y_c &= \frac{\sum y_i}{N};
\end{align*}
\]

the orthogonal distance of a given point from an axis through the centre, at an arbitrary angle \( \alpha \), is

\[
d_i = u_i \sin \alpha - v_i \cos \alpha,
\]

with \( u_i = x_i - x_c \) and \( v_i = y_i - y_c \). The main axis is defined by the condition that the sum of the squares of all distances is a minimum. This applies if the first derivative:

\[
\frac{\partial}{\partial \alpha} \sum d_i^2 = \sum \left( (u_i^2 - v_i^2) \sin 2\alpha - 2 u_i v_i \cos 2\alpha \right)
\]

is zero and if the second derivative

\[
\frac{\partial^2}{\partial \alpha^2} \sum d_i^2 = 2 \sum \left( (u_i^2 - v_i^2) \cos 2\alpha + 2 u_i v_i \sin 2\alpha \right)
\]

is positive. Therefore, from Eq. 4,

\[
\sin 2\alpha \cos 2\alpha = \tan 2\alpha = 2 \sum u_i v_i / \left( \sum u_i^2 - \sum v_i^2 \right)
\]

and, expressed in terms of \( x \) and \( y \) and optimized for computational efficiency,

\[
\alpha = 1 / 2 \tan^{-1} \frac{2(\Sigma x_i y_i - \Sigma x_i \Sigma y_i) / N}{(\Sigma x_i^2 - (\Sigma x_i)^2) / N - (\Sigma y_i^2 - (\Sigma y_i)^2) / N};
\]

add 90° to \( \alpha \) if Eq. 5 is negative.

The innermost program loops thus become:

for \( x = \) left margin to right margin

for \( y = \) top margin to bottom margin

if the pixel at \( x, y \) is set

then accumulate \( N, \Sigma x, \Sigma y, \Sigma x^2, \Sigma y^2 \) and \( \Sigma xy \).

(4) once for each frame, solve equations and apply geometry corrections

(5) display and store results and control stimulus as required.

2.5. Hardware

The host computer is 486-based and running at a clock rate of 50 MHz. The operating system is Microsoft DOS®.
stored as odd and even frames in separate buffers in video memory which can be processed in alternation. The resulting scheme of collecting and processing alternate frames, as a function of time, is detailed in Fig. 5.

Shortly before a vertical synchronization pulse ($t = -1$ ms) the even frame $i$ is exposed by a strobe flash, and a complete image is available in the sensor array of the frame transfer camera. The image information is instantaneously transferred to the storage array in the camera at $t = 0$ and then serially downloaded to video memory of the frame grabber in analog form, during the following 20 ms. At $t = 20 - 1$ ms, the odd frame $i$ is exposed. At $t = 20$ ms processing of the even frame $i$ by the graphics processor can begin. The cycle closes with exposure of the even frame $i + 1$ at $t = 40 - 1$ ms and the passing of the result for the even frame $i$ to the host, shortly after $t = 40$ ms.

To keep up with the incoming video data stream, a given frame of video information in memory, once it is complete, must be processed within 20 ms. During that period, the next frame must be downloaded into a separate area in video memory, to avoid overwriting of the frame which is being processed. Coincidentally, standard interlaced PAL video, a default option of the video interface, is preadapted to the requirement for separate storage of alternate frames. The output of the camera (which is operated in non-interlaced mode to avoid the half pixel vertical offset between frames which would occur otherwise) is memory from the host, necessary for example for the calibration steps, under mouse control (Fig. 6h).

2.6. Interlaced, pipelined video recording and processing

Fig. 5. Sequence of events during image processing. For further details see text.

Fig. 6. Steps in video image processing. (a) fly’s head en face; (b) fly with reflection foil marker on forehead; (c) suprathreshold pixels of marker and highlights; (d) image after remapping of suprathreshold pixels; (e) image after elimination of highlights by defocussing; (f) coarse scan to define window around marker for fine scan; (g) fine scan to compute centre and axis of marker; (h) calibration of magnification and alignment.
2.7. Processing: localization of the marker

To detect the marker optimally, the video threshold has to be set such that it is approximately halfway between the maximum brightness value of the marker and that of its surroundings. This is facilitated by displaying the camera signal on an oscilloscope which is triggered by the vertical synchronization pulses. After setting a threshold that slices the image such that the marker is above threshold (Fig. 6c, output of LUT, above threshold pixels shown as 100%, below threshold as 0%), it becomes obvious that some highlights may be still above threshold. They are small but far from the marker, resulting in a large error, meaning that a way must be found to remove them. As a prerequisite, it is necessary to make the threshold settings more easily visible and superimpose them on the image, while manually adjusting focus and threshold. To that end, the LUT is set up in such a way that input pixels with value zero are mapped as a small non-zero value (1 in the 8 bit range of 0.255). Other, below-threshold pixels are unchanged, but above-threshold pixels are mapped as zero. The result of this is that, merely by use of the LUT and without processing time penalty, the above-threshold areas appear black but are surrounded by fairly bright pixels (Fig. 6d). Assigning zero to above-threshold pixels also makes, during actual processing, testing for set pixels faster (assembly instruction: Branch on Zero).

It was found that an adequate method of removing highlights consists of optical low-pass filtering, by slightly defocussing the image (Fig. 6e). The focal plane is adjusted to be in front of the head, because defocussing in the opposite direction could produce new highlights from the body of the fly.

With the optimization described so far, processing a single pixel, in the tightest possible loop, requires 4 µs, allowing the processing of approximately 5000 pixels in the 20 ms available. That is a factor of 12 less than the total number of pixels on a screen, if a 256 X 256 pixel area is used. Therefore, scanning a complete image is not possible. However, as the marker only takes up a small fraction of the screen, it is sufficient to scan only a window within the image that is big enough to fully contain the marker. The prerequisite for this is knowledge of the approximate position of the marker.

One way to accomplish that could consist of finding the marker once and then anticipating its position in the next frame. Preliminary tests showed unambiguously that the amplitude of head movements between frames can be too large and their direction too unpredictable to allow that approach. Instead, an initial estimate of the position of the marker was made for each frame by a coarse scan, during the first half of each frame duration: every fifth pixel in the image (illustrated, for the purpose of clarity, by white dots in Fig. 6f) was tested and the coordinates of the centre of gravity at that resolution were calculated. A 55 X 55 pixel window was then defined around that point, shown by the square in Fig. 6f. The remainder of the frame duration was then sufficient to rescan that window with 1 pixel resolution and to calculate centre of gravity and angle as indicated in Fig. 6g.

2.8. Geometry corrections

During actual data acquisition, the steps in Fig. 6f,g are repeated continuously at the video rate, of course without actually drawing dots or lines on the screen. However, the marker coordinates have to be converted to the coordinates of the apex of the head axis and the marker axis has to be corrected for deviation from the vertical axis of the head. Also, the size of the head must be known to allow scaling of displacements to angles. To obtain this information, once only at the beginning of each experiment, an image as in Fig. 6g was frozen. Then, the operator, using a mouse, defines the coordinates of three well-defined points on the head, the apices of the eyes and the tip of the labrum (connected by V shape in Fig. 6h). This procedure relies upon the assumptions (1) that flies of slightly different size have similar head proportions, and (2) that flies do not shift the center of head rotation appreciably. Both conditions are met within the given limits (see Section 2.3).

2.9. Overall structure of software

A standard handshake procedure is used to synchronize processing. The vertical synchronization signal sets a bit in one of the frame grabber registers. This causes the program running in the GSP to initiate processing of a new frame. The program, written in native assembly code, accumulates the required terms in Eqs. 1-3 into GSP registers; at the end of the process, before the end of the frame, the GSP routine transfers the results to locations in video memory known to the host and raises a data ready flag, the host software, written in Pascal, reads the results, does the remaining calculations of marker coordinates and angle, as well as the geometry corrections. Triggered by the vertical synchronization pulses, the host also writes to the DACs controlling the angular positions of fly and pattern, reads the ADC monitoring the actual positions, updates a graphics display an the host monitor which shows, as a function of time, pitch, yaw and roll angles of the fly's head as well as the angular positions of the fly's trunk and the pattern (see Figs. 8-10), and stores data for further analysis.

3. Results and discussion

The performance criteria of the measuring procedure were specified with respect to the requirements of insect head/eye movements and gaze control: (a) resolution 1-2°.
(b) signal-to-noise ratio at least 5:1 (full scale), (c) deviation from linearity less than 5% over the working range, and (d) minimal crosstalk between signals of different axes.

For the angular ranges involved, the trigonometric calculations present no limit to accuracy. However, several physical factors tend in concert to degrade the performance: inadequate shape and contrast of the marker, inhomogeneous and varying illumination, incorrect scaling of head measures, and, foremost, inaccurate alignment of the fly with respect to the camera and the stimulator axis, especially when the fly is rotated.

Consequently, the accuracy of the procedure cannot be stated generally; it depends upon the care with which each of these factors is adjusted. Therefore we demonstrate the performance of our procedure in two steps: (1) some 'standard case' examples of technical performance tests (Fig. 7), and (2) realistic; experiments with live flies experiencing different stimuli (Figs. 8-10).

### 3.1 Technical performance

When a dead and dried fly carrying a marker on its forehead is mounted instead of a live fly, the technical performance can be tested under near experimental conditions but without interference from the fly's behavior. Fig. 7a shows, with the fly held stationary, that the records of all angles are stable over time and have a peak-to-peak noise level of 2° in this example but usually less than 1°. Hence the signal-to-noise ratio of the recordings is at least 10 for yaw and pitch, and 45 for roll, i.e., well beyond the required values.

When the dead fly's body is rotated at constant speed, and the monitoring of the fly's body position is disabled, the measurement of the head roll angle can be directly demonstrated (Fig. 7b). The sawtooth-shaped roll trace shows excellent linearity of the measurement over 360°; the increased noise in the middle range of the roll trace was caused, in this example, by a toothbelt ripple of the fly rotator and mechanical resonance of the fly holder. Yaw and pitch measurements, gained from marker displacements in the frontal plane, suffer from geometric distortions with increasing head eccentricity, and should be corrected by the corresponding trigonometric factors. At maximally 20° eccentricity (head motion range), however, this error amounts to an underestimation of the true angle of 0.4° at most, therefore we omitted this correction.

Calibrated horizontal and vertical displacements of a dead fly with a micromanipulator revealed no additional non-linearities (not illustrated). Taken together, the linearity of measurements is excellent for roll and good for yaw and pitch.

Despite the full rotation of the dead fly in Fig. 7b there is less than 1% crosstalk from the roll signal to the yaw and pitch signals. Conversely, displacing a dead fly horizontally or vertically yields no measurable crosstalk in the roll signal (not illustrated). This clear separation is routinely obtained if the fly's head axis coincides with that of body rotation. Live flies may hold their head somewhat obliquely during alignment which then causes crosstalk during paraxial rotations. Flies can be induced to reset their head accurately to its resting position by providing tarsal contact. This way crosstalk can be kept below 2% even if the fly is rolled. Without rotation of the fly's body, crosstalk is usually negligible.

Compensatory head movements of flies also depend upon the sensation of body turns through the halteres (Tracey, 1975; Sandeman and Markl, 1980; Hengstenberg, 1988). In order to measure the effect of haltere inputs on head position, it is necessary to turn the body and still
measure the excursions of the head, relative to the body. This means that the fly's body position has to be subtracted from the head position. The result of that operation is shown in Fig. 7c, again with a dead fly. The large transient in the roll trace is caused by the fact that the ring potentiometer which monitors the body position has a narrow region of undefined output at ± 180°. This position corresponds, however, to the fly flying upside down and is not used in most experiments. The non-zero slope of the roll trace in Fig. 7c is caused by a scaling error in the conversion of the potentiometer voltage to angle. It can be minimized by adjustment of the conversion factor.

3.2. Experiments with live animals

Previous experiments have shown that flies use visual and mechanosensory cues to sense their motion in space and to generate corrective flight manoeuvres as well as compensatory head turns (review Hengstenberg, 1993). It is also known that flies can turn their heads about all three principal body axes (Strausfeld et al., 1987). It was not clear, however, whether the turning responses are coaxial with the stimulus rotation or whether they occur around an oblique axis. In this case, a considerable component of the response on axes other than the stimulus (roll) axis could be expected (Nalbach and Hengstenberg, 1994).

By simultaneous recording of the three principal components of head rotation in response to visual and mechanosensory roll stimuli it can be demonstrated that both stimulus modalities elicit coaxial responses with little crosstalk to the other axes of head turning. (Figs. 8 and 9).

Fig. 8 shows head turns induced by visual input, caused by the roll motion of a striped pattern, sinusoidally rotated around a stationary fly during flight. The head roll position is in phase with the pattern position; a peak-to-peak excursion of the pattern by 110° evokes a head excursion of 70°.
In addition to linearly following the pattern, the roll response also contains components which are not correlated with the pattern and are generated spontaneously by the fly during flight. The yaw and pitch movements are less correlated with the stimulus, if at all. They are also largely independent of each other.

To obtain the result in Fig. 9, the fly was sinusoidally rotated around the roll axis, in visually featureless surroundings. Under those conditions, the compensatory response is mediated by the halteres. The head movements, relative to the body, partially compensate for the imposed body roll: a peak-to-peak body movement of 120° evokes a head movement of 54°, in opposite phase. Again, additional roll movement components and independent pitch and yaw movements are evident.

The neural subsystems for visual and mechanosensory roll sensation have different frequency characteristics: visual motion perception is optimal at moderate roll speeds, whereas self-motion perception through the halteres is most sensitive at higher turning velocities (Hengstenberg, 1993). Hence the two sensory channels complement each other and provide the fly with a sufficiently wide range of motion perception (Hengstenberg et al., 1986).

As the stimuli were perfectly regular, it is possible that the results shown in Figs. 8 and 9 do not truly represent the quality, or closed loop gain, of reflexive gaze control: the fly might predict, after a few stimulus cycles, where to position its head properly. Such predictive stabilization of gaze is well known from human subjects tracking a sinusoidally moving target (Stark et al., 1962). Therefore, it is necessary to determine closed loop gain under conditions where the fly cannot predict the stimulus motion.

Fig. 10 shows an example where a fly is randomly rolled in optically structured surroundings. Both visual and mechanosensory channels are stimulated and elicit head turns (middle trace: head on trunk) in opposite direction to the imposed roll stimuli (lower trace: trunk in space). These head movements reduce the angular misalignment of the head in space and its angular velocity relative to the surroundings but head stabilization is by no means perfect (upper trace: head in space). This is consistent with the notion that, in free flight, simultaneously executed steering manoeuvres by the wings would supplement the corrective head turns and increase the overall gain of the feedback loop. The higher gain at high fluctuation rates is consistent with previous results (Hengstenberg et al., 1986).

4. Conclusions

We have developed and successfully tested a method for automatic real-time logging of fly head movements, simultaneously in the three axes of head mobility. The method requires only standard equipment and moderate user skills. It impairs the physiological condition of the fly only marginally; individual animals could be repeatedly studied over 6 weeks in the laboratory which is presumably more than their normal life span in the wild.

The method has several major advantages and, of course, also a few minor disadvantages. The disadvantages are:

(1) The fly has to be held in place and can only be turned about the optical axis of the measuring camera.

(2) Generally, movements of the marker must be restricted to the image plane and be within the range of focal depth.

(3) If crosstalk from the roll axis on the other axes is a concern, the fly has to be very carefully aligned with the camera and the stimulus motor axis. If not, the precision requirements for alignment are much less demanding.

(4) The total duration of image processing (40 ms) imposes a limit on the response time for closed-loop applications; this might be too slow, given the fly’s ability
to generate rapid head turns (Land, 1975; Hengstenberg et al., 1986).

However, advantages of the method are:

(1) It allows to measure, record and display all components of arbitrary head turns of the fly with sufficient accuracy and signal-to-noise ratio.

(2) Individual flies can easily and repeatedly be observed for extended periods of time. In an extreme case, about 85% of a non-stop flight lasting almost 6 h could be recorded, meaning that almost 10^6 video frames have been evaluated to document this flight. The remaining 15% were spent for typing comments and storage of the data. If necessary this gap could be reduced to less than 1%. This allows studies to be made which would be prohibitively time-consuming with manual or semiautomatic evaluation of video images.

(3) By the same token, responses to small and/or aperiodic stimuli can be captured, and for periodic stimuli large numbers of trials per experiments are within reach, allowing signal averaging to reduce the effect of random fluctuations of responsiveness and other noise.

(4) Finally, the principle of the method is such that it is not restricted to the study of head movements in insects. With appropriate modifications it can be adapted to the measurement of a large variety of small movements.

Acknowledgements

We wish to thank Mrs. K. Bierig for her skillful assistance and expertise with the experiments, Prof. K.G. Götz for the fast algorithm to determine position and orientation of the marker, and Mr. R. Feiler for the adaptation of the programs to the programming environment used in this institute (Borland Pascal 7.0).

References


