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Variability of blowfly head optomotor responses

R. Rosner[1,2,*], M. Egelhaaf[1], J. Grewe[3] and A. K. Warzecha[1,2]

[1]Lehrstuhl für Neurobiologie, Universität Bielefeld, Bielefeld, Germany, [2]Psychologisches Institut II, Westfälische Wilhelms-Universität Münster, Münster, Germany and [3]Abteilung Biologie II, Ludwig-Maximilians-Universität München, München, Germany

*Author for correspondence (e-mail: ronny.rosner@uni-bielefeld.de)

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SUMMARY

Behavioural responses of an animal are variable even when the animal experiences the same sensory input several times. This variability can arise from stochastic processes inherent to the nervous system. Also, the internal state of an animal may influence a particular behavioural response. In the present study, we analyse the variability of visually induced head pitch responses of tethered blowflies by high-speed cinematography. We found these optomotor responses to be highly variable in amplitude. Most of the variability can be attributed to two different internal states of the flies with high and low optomotor gain, respectively. Even within a given activity state, there is some variability of head optomotor responses. The amount of this variability differs for the two optomotor gain states. Moreover, these two activity states can be distinguished on a fine timescale and without visual stimulation, on the basis of the occurrence of peculiar head jitter movements. Head jitter goes along with high gain optomotor responses and haltere oscillations. Halteres are evolutionary transformed hindwings that oscillate when blowflies walk or fly. Their main function is to serve as equilibrium organs by detecting Coriolis forces and to mediate gaze stabilisation. However, their basic oscillating activity was also suggested to provide a gain-modulating signal. Our experiments demonstrate that halteres are not necessary for high gain head pitch to occur. Nevertheless, we find the halteres to be responsible for one component of head jitter movements. This component may be the inevitable consequence of their function as equilibrium and gaze-stabilising organs.

Key words: optomotor response, variability, behavioural state, halteres, head movements, arousal state.

INTRODUCTION

The nervous system of an animal enables it to adjust itself to environmental changes by producing different behavioural activities. A particular behavioural activity can change in amplitude depending on the stimulus strength. In addition, when experiencing the exact same stimulus repeatedly, an animal does not respond in the same way each time. Several noise sources arising at different levels of a neuronal pathway can, in principle, cause this variability, e.g. sensory noise such as the phototransduction process in photoreceptors (Rodieck, 1998), synaptic noise due to the probabilistic nature of quantal transmitter release, as well as electrical noise introduced by the stochasticity of the opening and closing of ion channels (Faisal et al., 2008; Johnston and Wu, 1995). Moreover, behavioural responses may depend on the animal's internal state. For instance, a car driver, being in a hurry, will possibly undergo a hazardous overtaking manoeuvre that the same person facing the same situation yet being in a relaxed mood would not. There are several possibly less spectacular but nevertheless interesting examples of behavioural gain changes subject to the animal's behavioural state. For example, when hearing a male's calling song, female crickets will increase the gain of auditory steering responses within the next 2–5 s (Poulet and Hedwig, 2005). In addition, locomotion *versus* resting represent behavioural states, i.e. whether an animal is actively moving or not was found to affect signal processing in nervous systems as well as the gain of behavioural responses (Gilbert and Bauer, 1998; Heide, 1983; Hengstenberg et al., 1986; Horn and Lang, 1978; Nolen and Hoy, 1984; Reichert et al., 1985; Sillar and Roberts, 1988; Staudacher and Schildberger, 1998). Many of these studies were carried out on insects because of their relatively small number of neurones involved in producing a particular behavioural activity. In combination with an electrophysiological accessibility of neurones at several processing stages, insects provide the opportunity to unravel general mechanisms of neuronal information processing. Flies, despite their small brains, are capable of executing virtuosic flight manoeuvres, requiring that the sensory information is reliably processed and transformed into motor behaviour (Egelhaaf and Borst, 1993; Frye and Dickinson, 2001; Hengstenberg, 1993; Schilstra and van Hateren, 1998). The variability of visual information processing in the nervous system of flies was the subject of many studies in the last few years (Borst and Theunissen, 1999; Egelhaaf and Warzecha, 1999; Egelhaaf et al., 2005; Grewe et al., 2003; Grewe et al., 2007; Haag and Borst, 1997; Juusola et al., 1994; Ruyter van Steveninck and Laughlin, 1996; Ruyter van Steveninck et al., 2001; Warzecha and Egelhaaf, 1999; Warzecha and Egelhaaf, 2001; Warzecha et al., 1998; Warzecha et al., 2000). Compared with the detailed characterisation of the variability of the neuronal responses in the fly's visual motion pathway, relatively little is known about the consequences of this variability for behavioural performance. Turning responses induced by large-field visual motion stimuli during tethered flight are highly variable compared with the variability of motion-sensitive inter-neurones providing the visual input to the flight motor (Warzecha and Egelhaaf, 1996). Moreover, flies occasionally omit turning manoeuvres towards an object (Zanker et al., 1991), although response failures have not been observed at the level of the respective motion-sensitive neurones mediating object fixation (Egelhaaf, 1985). Response failures at the behavioural level indicate the action of some kind of gate downstream to the visual system that introduces variability of the motor output.

In the present study, we investigate the variability of optomotor head movements of blowflies, which counteract retinal image slip and are likely to play a role in stabilising the gaze (Hengstenberg, 1984; Hengstenberg, 1991; Hengstenberg, 1993; Hengstenberg et al., 1986; van Hateren and Schilstra, 1999). Visually induced head movements may be more reliable than yaw torque responses because they fine tune gaze-stabilising body movements. In certain phases of free flight, the fly's head is more stable than its thorax (van Hateren and Schilstra, 1999). We monitored the head movements of tethered flies with high-speed cinematography while the animals were stimulated with visual motion. We found that the amplitude of optomotor head pitch responses are highly variable and that part of this variability can be attributed to two different states of behavioural activity that differ in optomotor gain. However, not only does variability across behavioural states exist but it also exists within a given state. The variability of the optomotor response amplitude is much higher in the high gain state than in the low gain state. Nevertheless, the signal-to-noise ratio (SNR) in the high gain state is not smaller than the ratio in the low gain state because of the larger optomotor response amplitude in the high gain state.

For fly head movements, a particularly unique mechanism of gain control was proposed. Halteres, the evolutionary transformed hindwings of dipterans, were suggested to provide a gain-modulating signal (Gilbert and Bauer, 1998; Huston, 2005; Sandeman, 1980). The main function of the halteres is to serve as an equilibrium and gaze-stabilising organ when the fly moves around (Dickinson, 1999; Nalbach and Hengstenberg, 1994; Pringle, 1948). They oscillate when the fly walks or flies (Sandeman and Markl, 1980). Their base is equipped with a large number of mechanoreceptors that detect deflections out of the main beating plane of the halteres, which occur when the fly rotates while moving (Chan and Dickinson, 1996; Gnatzy et al., 1987; Pringle, 1948). However, not only are these deflections encoded by the mechanoreceptors but the basic oscillating rhythm is as well (Fayyazuddin and Dickinson, 1996; Huston, 2005; Pringle, 1948). It is this signal that could serve as a gain modulator and could be responsible for the head jitter movements that we observe when the animals have a large optomotor gain. We will show that the mechanosensory, reafferent signals mediated by the halteres cannot, on their own, account for the dramatic increase of optomotor head pitch in the high gain state.

MATERIALS AND METHODS

Preparation

The experiments were carried out on 15 female blowflies (*Calliphora vicina* Robineau-Desvoid) that were up to five days old, and which were taken from the laboratory stock. The flies were briefly anesthetised with CO_2 or immobilised by cooling them down. Flies were attached to a holder, which was glued to the thorax with a drop of bees wax. Legs and wings were cut and the remaining stumps were fixed with bees wax to prevent vibrations of the animal caused by intended wing beat or leg movements; otherwise these vibrations would have deteriorated our analysis of stimulus-induced head movements of the flies. To facilitate the detection of head movements, two dots were painted on the ventral side or four dots on the lateral side of the fly's head (paint: Universal Abtönpaste, Kemper and Company, Mittenaar, Germany), depending on whether the fly was filmed ventrally or laterally (see below). Throughout the paper, these dots will be called markers. These markers reflected infrared light and enabled us to film head movements without light that is visible to flies. The tips of the halteres were also marked in this way. When flies were filmed ventrally the ventral prothorax was also marked. When filming the fly laterally, two of the four markers on the fly's head were painted on the eye (Fig. 1); however, this did not constrain the visual stimulation. The visual stimulus was applied to the frontal visual field of the fly, while the markers covered a small area responsible for acquiring caudo–lateral visual input.

We evaluated data of four flies that were filmed ventrally (Figs 10–13) and 11 flies that were filmed laterally (Figs 3–9, 14). We filmed ventrally when it was necessary to detect movements of both halteres, and we filmed laterally when it was more important to resolve head pitch angles as well as haltere oscillation frequencies of the one haltere that could be monitored in this way. The pitch responses of three or two of the flies filmed laterally were analysed before and after removing or immobilising the halteres, respectively (example experiment in Fig. 14).

Visual simulation

The fly was positioned in front of a CRT-Monitor (Vision Research Graphics, Durham, NH, USA) with a frame rate of 240 Hz and a resolution of 640×480 pixels. In the screen centre, one pixel was 0.18×0.18 deg. in size as seen by the fly. The whole screen was used to present the visual stimulus, spanning an elevation from –25 deg. (ventral) to +45 deg. (dorsal) and an azimuth from –45 deg. to +45 deg. with respect to a straight head position of the fly (0 deg., 0 deg.). The stimulus was programmed and presented utilising the Visage stimulus generator (Cambridge Research Systems, Cambridge, UK), Matlab (The MathWorks Inc., Natick, MA, USA) and a standard PC.

We used a random dot pattern as the visual stimulus. The pattern consisted of 40 randomly positioned dots of 2 deg. horizontal and 2 deg. vertical extent. Individual dots were spaced with a minimum distance of 8 deg. During each trial, the stimulus moved downwards for 200 ms at 168 deg. s^{-1}. We chose the stimulus velocity of 168 deg. s^{-1} to elicit large optomotor responses and thus to minimise the relative influence of the noise of our technical equipment when estimating the variability of the optomotor responses. Possible noise sources are for instance minimal vibrations of the experimental setup as well as random intensity fluctuations of individual pixels on the camera chip.

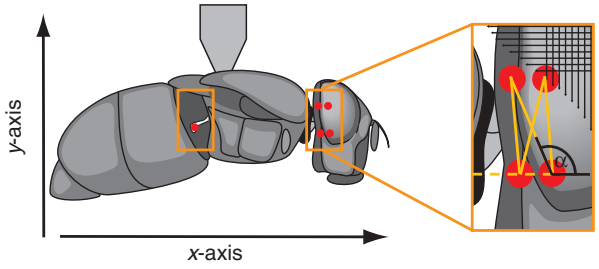

Fig. 1. Scheme of a laterally filmed tethered fly with the legs and wings removed. Haltere and head were labelled with infrared-light-reflecting markers (here red dots) to enable evaluation of their movements. For analysis of the image data, two regions of interest (ROIs), illustrated as orange rectangles, were positioned upon the haltere and head, respectively. Haltere position was determined as outlined in the text. In the zoomed ROI, it is indicated how the head pitch angle was determined. The grid in the upper right corner of the zoomed ROI illustrates pixel columns (in *y*-direction) and rows (in *x*-direction) in the image. Four straight lines interconnect the markers. α illustrates the angle subtended by one of these lines with the horizontal. The mean angle of the four lines with the horizontal determines the pitch angle of the head in this particular image frame. (Scheme of fly courtesy of Christian Spalthoff.)

Data acquisition

The fly was filmed at 500 Hz using a CMOS CameraLink® camera (LOGLUX i5 CL, Kamera Werk Dresden, Dresden, Germany). To achieve a frame rate of 500 Hz, the read-out window of the sensor chip was restricted to 270×147 pixels of the available 1280×1024 pixels. The exposure time was set to 0.275 ms. CameraLink® signals were converted to low voltage differential signals (LVDS/RS644) using an IMPERX Adapt_A_Link™–BCL converter (IMPERX, Boca Raton, FL, USA), readable for the IMAQ PCI-1424 frame grabber (National Instruments, Austin, TX, USA). The image data were acquired using a standard PC and the data acquisition software idVIEW (Aspect Systems, Dresden, Germany). The fly was illuminated by near-infrared light emitting diodes (LEDs) (TSFF5200, Vishay, Selb, Germany) with a peak wavelength of 870 nm, which is beyond the spectral sensitivity of *Calliphora* photoreceptors (Hardie, 1979). The spectral sensitivity of the camera ranged up to 1000 nm. The paint used to mark certain parts of the fly's body reflected infrared light (see above).

Experimental procedure

In each experiment, the fly experienced repetitions of the same stimulus for about two hours. For the six flies that were used to study the reliability of the optomotor responses, no data were evaluated from at least the first 45 min of the experiment. During this period, we enabled the fly to get used to the setup and thus prevent a possible impact of transient response changes on the variability of the optomotor responses. One stimulus sequence is called a trial throughout this paper (see Fig. 2 for an illustration of a stimulus sequence). The entirety of all of the trials presented to one fly will be called an experiment. Each trial contained a 100–300 ms reset stimulus shown on the monitor to reset the head of the fly to its reference orientation after the preceding trial. As reset stimulus, an upward moving dot pattern or flicker was used. A dot pattern was then presented for 1000–1200 ms as a still image before moving downwards for 200 ms at a velocity of 168 deg. s^{-1}.

The camera was triggered 200 ms before motion onset. 190 images (i.e. 378 ms) were acquired and evaluated per trial, with frame 101 being acquired at pattern motion onset (defined as 0 ms). A new trial was presented every 10 s. After each experiment, the temperature was measured at the position of the fly. Temperatures ranged between 29 and 35°C as a consequence of the infrared illumination needed for filming head movements. Such high temperatures also occur on warm sunny days when the fly is active.

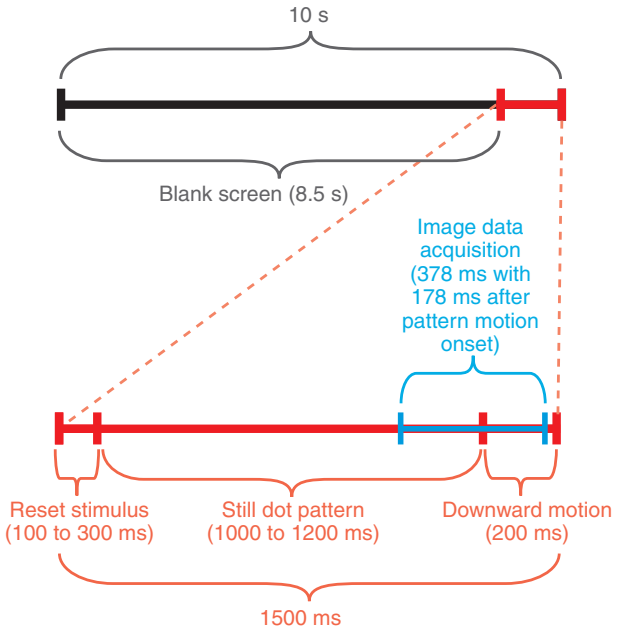

Fig. 2. A single stimulus sequence (trial) and inter-stimulus pause. Trials (red) were separated by pauses with a blank screen (black). This sequence was experienced several hundred times by the fly in each experiment. One trial consisted of a reset stimulus shown on the monitor, stimulating the fly to reposition its head in a starting position. Subsequently, a random dot pattern first remained motionless and then moved downwards, inducing the optomotor head pitch response analysed in the present study. Image data were acquired for 378 ms (blue) starting 200 ms prior to pattern motion onset.

Data analysis

Data were analysed using Matlab. To evaluate head, haltere and thorax movements, regions of interest (ROI) in the images were adjusted by visual inspection so that they contained the haltere, head- or thorax markers (see Fig. 1 for scheme of a laterally filmed fly). Markers were detected from the background by a threshold operation. The *x*- and *y*-coordinates of the centre of brightness of each marker were determined in each image. The *x*-coordinate describes a position along the longitudinal axis of the fly, and the *y*-coordinate along the transversal axis (ventrally filmed flies) or the vertical axis (laterally filmed flies). By applying the centre of brightness calculation, we achieved sub-pixel accuracy and were able to detect movements of less than 1 µm.

Evaluation of head pitch movements when the fly was filmed laterally

The four centres of brightness of the four markers painted on the head were interconnected by straight lines (Fig. 1). The angles, subtended by these lines, with the horizontal were averaged to obtain an angle representing the head orientation in the respective frame.

Evaluation of haltere movement when the fly was filmed laterally

As the halteres mainly oscillated along the *y*-axis, we only evaluated the elevation of the haltere to quantify its movement. Therefore, the ROI with the haltere marker was compressed to a single column in each frame by averaging within rows. Subsequently, the threshold was set to separate the marker from the background, and the centre of brightness was calculated leading to a *y*-value representing haltere elevation.

Evaluation of head pitch and thorax movements when the fly was filmed ventrally

Only the *x*-coordinate of one of the two centres of brightness calculated for the two markers on the ventral side of the head was used. We obtained basically the same results, no matter which of the two centres we chose. Head movements, measured as displacements along the longitudinal axis of the fly, could, in principle, also be caused by head translations instead of pitch responses. However, the displacements are in the order of magnitude we expected when taking the information about pitch angles into consideration as determined when filming the flies laterally. Hence, we will refer to the measured displacements as pitch movements although, strictly speaking, we did not measure pitch angles.

When evaluating thorax movements we also used only the *x*-coordinate of one of the two centres of brightness calculated for two markers that were painted on the ventral side of the thorax. Again, we obtained qualitatively the same results when the other centre of brightness was used.

Evaluation of haltere movements when the fly was filmed ventrally

The x- and y-components of the haltere positions in two successive images were used to calculate the speed of the projection of the haltere tip on the camera-sensor-chip:

$$Speed_i = \sqrt{(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2},$$

with x_i and y_i representing the haltere tip x- and y-position in image frame i, respectively.

Evaluation of head jitter when flies were filmed either ventrally or laterally

Flies sometimes spontaneously underwent high-frequency oscillations of the head, which we call head jitter (see Results). In order to separate the time intervals with and without head jitter, we removed stimulus-induced pitch movements and other low-frequency components by high-pass-filtering the time-dependent traces of head position with a 8th-order Butterworth filter with a cut-off frequency of 90 Hz. Subsequently, the absolute values and the square root of these pitch fluctuations were taken, because we found times with and without head jitter to be more easily identifiable after this operation. In particular, the two peaks in the histograms characterising time intervals with and without head jitter (Fig. 3, see below) are more distinct. Times with and without head jitter cannot be identified on a 2 ms timescale (the temporal resolution of the data) because this interval is too short to identify head jitter that had its strongest frequency component above 100 Hz and below 200 Hz and thus a cycle duration of more than 5 ms (Fig. 9). We found 20 ms to be a good trade-off between the need to include more than one oscillation period and the goal to assess head jitter on a fine timescale. Therefore, traces were subdivided into 20 ms bins. Within each bin, the sum of these values was computed that resulted from the head position traces after execution of the above-mentioned operations. The typical histogram of all the bin-sums of an entire experiment shows a bimodal distribution (see Fig. 3; Fig. 11B). The first peak of the distribution is composed of bins without conspicuous head jitter, and the second peak results from bins with pronounced head jitter. A threshold was determined between the peaks by visual inspection to separate bins with and without jitter in the following data analysis (see Results).

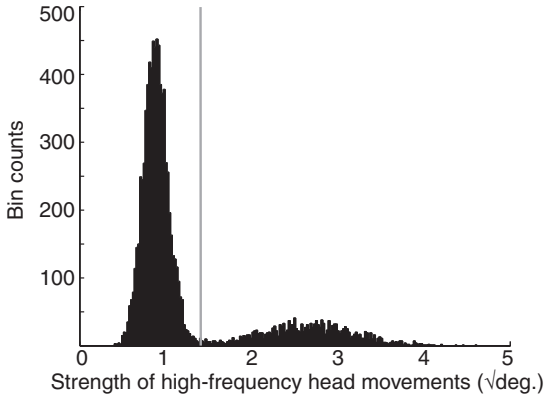

Fig. 3. Bimodal distribution of high-frequency head jitter (above 90 Hz). Frequency histogram of the strength of head jitter of one fly evaluated within 20 ms bins. All traces obtained from the fly were used for the histogram. The bimodal distribution indicates the existence of two distinct states of behavioural activity of the fly going along with little or no head jitter (left peak) and conspicuous head jitter (right peak), respectively. Between the two peaks a threshold value was set (grey vertical line) to classify data according to these two states.

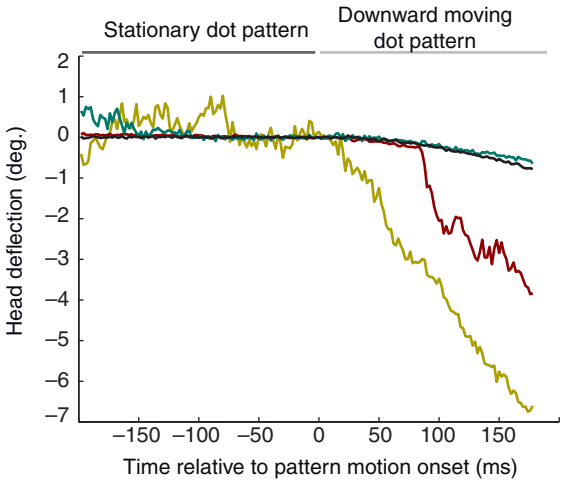

Fig. 4. Example traces illustrating head optomotor pitch and head jitter movements. Four example traces are shown with head jitter throughout the trial (yellow), no large amplitude jitter at all (black), one trace starting with head jitter and ending without jitter (green) and one trace starting without large head jitter but starting jitter within the trial (red). The traces were aligned to have zero mean in a 50 ms interval starting 42 ms before stimulus motion onset. When the fly shows conspicuous head jitter, the optomotor response is stronger than without jitter.

Particularities when analysing optomotor responses for the reliability analysis (Figs 5–8)

Although a reset stimulus (see above) was employed to shift the head back to a reference orientation, head orientations at the beginning of pattern motion varied between trials. In order to minimise a potential effect of variable head position at the start of pattern motion on the final pitch response, only a subset of trials was selected for further analysis. For the selected trials, the starting head orientation was required to fall within a range defined by the head orientations assumed by the fly when it was jittering with its head; for each of the traces with head jitter occurring throughout the first 200 ms of the trial, i.e. the time period before stimulus motion onset, the mean head orientation in the 20 ms interval preceding stimulus motion onset was determined. The standard orientation was defined as the median of these values across trials. We accepted only those trials for further analysis with a mean head orientation in the 20 ms interval that deviates less than 5 deg. from the standard orientation. Subsequently, the measured head orientation traces of all selected trials were aligned to have zero mean in a 50 ms interval starting 42 ms before stimulus motion onset. This procedure was applied to each fly separately.

We evaluated the mean pitch amplitude, the standard deviation and the ratio of both, i.e. the SNR for each fly for the last analysed image, i.e. 178 ms after stimulus motion onset. To check whether the head jitter itself increases the variability of the determined head pitch optomotor gain, we removed the jitter from the data by fitting a third-order polynomial to the head orientation curves, starting 10 ms after pattern motion onset, applying the Matlab function ‘polyfit’.

RESULTS

In response to visual downward motion, flies showed an optomotor following reaction by pitching their head downwards. The amplitude of these head deflections varied greatly in different trials. Large optomotor responses went along with head jitter, which can already

be observed before the onset of pattern motion. In traces with small optomotor responses, we observed no obvious head jitter, i.e. jitter that exceeded the small fluctuations also observed when filming a rigid object due to measurement noise and small vibrations of the setup. In part of the traces, the head jittered throughout the trial; in other traces the head started or stopped jittering sometime during the trial (Fig. 4). In order to quantify head jitter movements, we subdivided individual trials into 20 ms bins. Time bins of this size are large enough to capture the jitter activity of head movements while being small enough to provide information about the time course of head jitter on a relatively fine timescale. The histogram of the strength of head jitter from all of the time bins recorded from one fly reveals two distinct peaks (Fig. 3). This two-peaked histogram suggests that head jitter reflects two states of behavioural activity. Potential mechanisms that mediate head jitter will be discussed later in the paper.

Relationship between optomotor gain and head jitter

For a quantitative analysis of the relationship between head jitter and the amplitude as well as the variability of head optomotor pitch responses, we separated trials with large head jitter from those without head jitter. The separation was done by comparing the strength of the head jitter within each 20 ms bin with a threshold value (grey vertical line in Fig. 3). Trials with head jitter starting or stopping within one trial were omitted because we do not want to analyse state transitions in this section of our paper but rather characterise a given behavioural state. In Fig. 5 all remaining trials recorded in one fly are shown after separating them based on the occurrence or absence of head jitter movements. The amplitude of optomotor responses, i.e. head deflection, varies considerably (note the different scaling in Fig. 5A,B). Nevertheless, the gain of the optomotor pitch response is much higher when head jitter is large than when hardly any head jitter can be discerned as is illustrated for one single fly in Fig. 5 and corroborated by the time courses of the mean optomotor head pitch traces obtained from six different flies (Fig. 6A).

During head jitter or without head jitter, flies start pitching their head downwards approximately 22 ms or 27 ms, respectively, after stimulus onset. Moreover, the slope of the pitch response differs in both cases. When head jitter is large, the mean slope of head deflection is large but appears to decrease slightly in time whereas the mean slope of the time-dependent head deflection is small but gets steeper throughout the trial when there is no conspicuous jitter. Fig. 7A shows the pitch response amplitudes 178 ms after stimulus onset for the six experiments. It may seem arbitrary to choose this one time point for quantifying the head optomotor responses. However, head position at the end of the trial, on the one hand, ensures that clearly measurable responses are obtained even for the small optomotor responses observed when the flies did not jitter with their heads. On the other hand, head angular position results from integrating head velocity and thus reflects overall response strength. For each fly, the mean head pitch optomotor response was much larger when going along with head jitter than without head jitter. The mean optomotor pitch amplitudes at 178 ms after stimulus onset were 7–29 times larger with head jitter than without head jitter. Hence, as judged by head jitter and optomotor pitch movements of the head, the fly appears to assume two behavioural activity states. These two states will be termed high and low activity state, respectively, in the following text.

The above conclusions still need to be qualified. Before stimulus onset, the head angle was not constant but drifted in most flies to some extent (Fig. 6A). In many experiments during the high activity state, the heads of the flies were, on average, pitching slightly downwards before stimulus onset whereas in the low activity state, the head tended, on average, to pitch upwards before stimulus onset. Due to its tiny amplitude this upward drift is not detectable in Fig. 6A. These opposing drifts occurred even though the fly experienced the same reposition stimuli to reset the head orientation after the foregoing trial (see Materials and methods). The cause for the drift and its state-dependent direction is not entirely clear; obviously, there are after-effects of the preceding reset stimulation that differ for the high and low activity states. To test whether the larger optomotor gain in the high activity state may have resulted from this drift, we corrected the stimulus-induced pitch movements for the drift. Drift correction was accomplished for each trial separately by fitting a regression line to the data recorded before response onset (from 198 ms before to 8 ms after stimulus motion onset). For drift correction, the regression line was extrapolated to the end of the trial and subtracted from the respective curve. Fig. 6B shows the mean time-dependent head deflections for the drift-corrected trials in the two states. The drift before motion onset is largely eliminated, indicating that the fitted regression lines adequately represent the drift. Obviously, even after correction for a possibly sustained linear drift superimposed on the stimulus-induced response, the mean pitch responses of all flies during the high activity state are larger than the corresponding low activity responses (Fig. 6B; Fig. 7B). Hence, a larger optomotor response in the high activity state than in the low activity state is indeed caused by a different gain in the two states and is not an artefact of the drift in head position. In the following we will continue to present

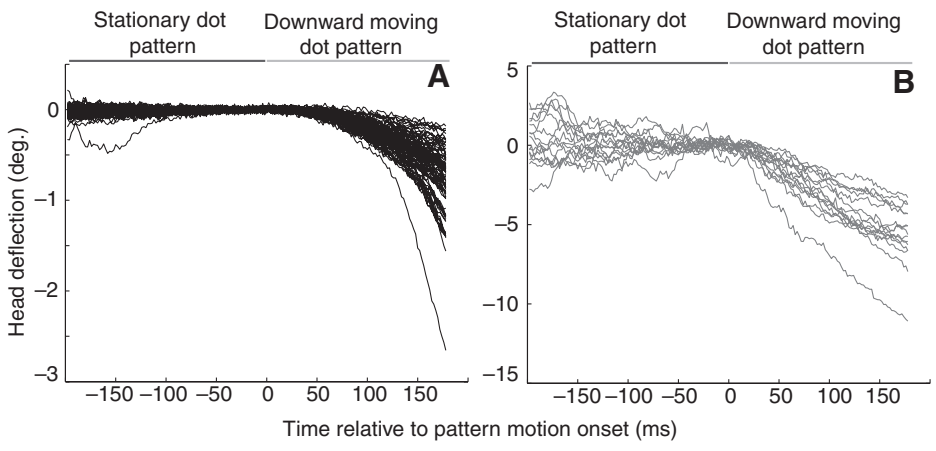

Fig. 5. Optomotor responses of one fly separated in traces without (A) and with (B) conspicuous head jitter. All traces of one typical experiment with, B, or without conspicuous head jitter, A, throughout the trial are shown. Visually induced head deflections are larger and more variable in amplitude when going along with conspicuous head jitter than without. Note the different scaling of the ordinates in A and B.

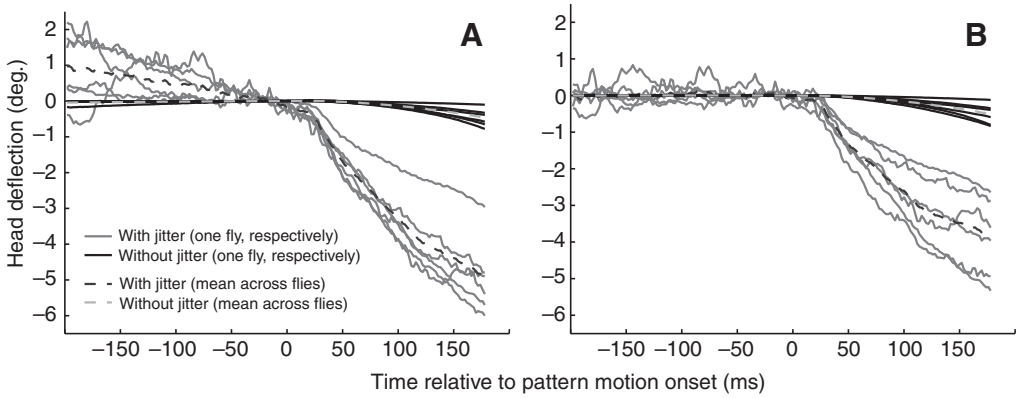

Fig. 6. For each fly the mean head deflection is shown for responses with and without conspicuous head jitter. Dotted lines illustrate the means across flies in the two states. (A) For each fly, as well as for the mean across flies, the visually induced head deflections are considerably larger when going along with large head jitter. Before motion onset, the head position is not stable but drifts consistently downwards/upwards for mean responses of trials with/without large head jitter. The upward shift in traces without conspicuous jitter is hardly visible due to the scaling. (B) Head drift was compensated for each individual trial before averaging. Head pitch responses are still larger when accompanied by jitter.

the results for the drift-corrected data as well as for the uncorrected data because, on the one hand, drift correction affects the results at least quantitatively. On the other hand, it is not possible to decide on the basis of our data whether the observed drift continues after stimulus motion onset and whether it does so in a linear manner. Determining results for corrected and uncorrected data at least allows us to estimate the range of possible outcomes.

The gain of the optomotor response is calculated as the ratio of the head angular velocity and the pattern velocity for the initial phase after head motion onset (i.e. between 40 ms and 80 ms). The optomotor gain amounts for the high activity state, on average (±standard deviation), to 0.18±0.06 and 0.21±0.05 for the drift-corrected and uncorrected data, respectively. The optomotor response gain for the low activity state is more than one order of magnitude smaller (about 0.01±0.006).

Reliability of optomotor pitch responses within the two activity states

In the high activity state not only was the optomotor gain larger but the variability of the optomotor pitch response was also larger. The variability is quantified as the standard deviation of the individual pitch angles of a fly from the corresponding mean response (error bars in Fig. 7).

If head jitter itself increased the standard deviation considerably, the elevated variability in the high activity state was an artefact resulting from our sorting algorithm, which classifies trials with head jitter as high activity responses and those with a nearly motionless head as low activity responses. However, a third-order polynomial fit to the curves that starts at 10 ms after stimulus onset and effectively smoothes out the jitter, confirmed that optomotor gain variability and not head jitter caused the large standard deviations across trials in the high activity state (data not shown).

The mean responses and the response variability on their own do not reveal much about the reliability of the behavioural responses. Instead, both need to be related in some way. We therefore determined the SNR as a measure of the reliability of the responses. It was calculated as the ratio of the mean pitch amplitude and the standard deviation 178 ms after stimulus onset. Fig. 8 shows the SNRs for the six flies in the two activity states with and without drift correction. The SNRs of the responses uncorrected for drift is

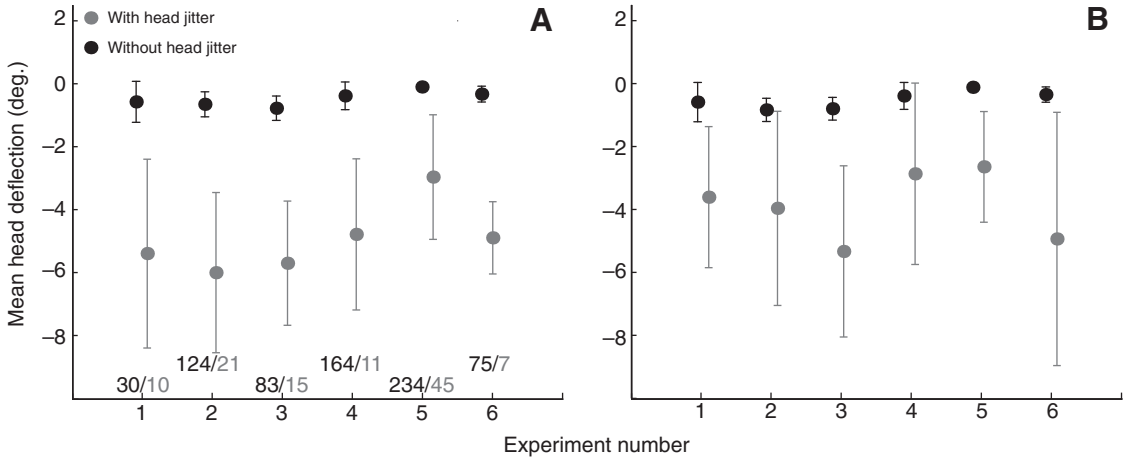

Fig. 7. Mean amplitude of the head pitch response for each of six flies. Head pitch was determined at the end of the trial, i.e. 178 ms after stimulus motion onset. (A) Before and (B) after drift compensation. The numbers below data points denote number of individual trials without (black) and with (grey) head jitter. Error bars denote standard deviations. For each fly, head pitch is larger when going along with conspicuous head jitter irrespective of drift compensation.

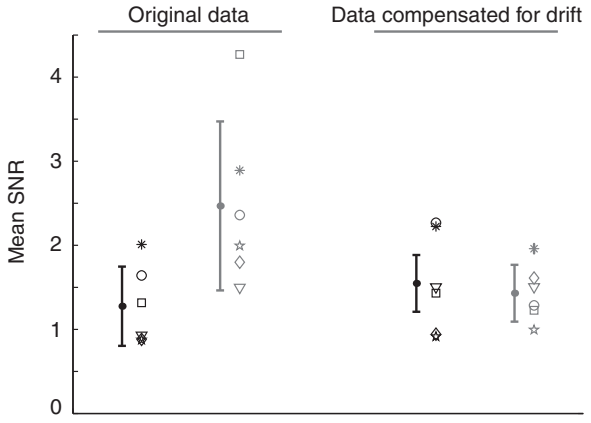

Fig. 8. Mean signal-to-noise ratios (SNRs) at 178 ms after stimulus motion onset. The SNR in the high activity state is higher and significantly different from the low activity state SNR for the uncorrected drift data (α=0.05). The SNR in the high activity state is not significantly different from the low activity state SNR for the drift corrected data (α=0.05). Symbols represent individual experiments. Dots and error bars represent means across flies and standard deviations, respectively.

significantly higher in the high activity state than in the low activity state (Welch's test with α=0.05). For the drift-corrected curves, however, the SNR is not significantly different between the high and the low activity states (Welch's test with α=0.05). As outlined above, it cannot be definitely determined on the basis of our data whether the assumptions are satisfied that underlie drift compensation. Therefore, it is not possible to definitely conclude whether or not the SNR of the optomotor pitch response is improved in the high activity state compared with the low activity state. Nonetheless, it can be concluded, that the SNR did not decline in the high activity state.

We ensured that the following two possible artefacts did not affect our results. (1) In some experiments the pitch movements in the high activity state showed some saturation towards the end of the evaluation period (Fig. 6A). This could, in principle, reduce optomotor response amplitudes and their standard deviations. To make sure that we did not misjudge the SNR for this reason we determined the SNR for all time points following stimulus onset when saturating behaviour evidently did not occur. Our conclusions concerning the SNR in the high and low activity states hold for all of the time points following the onset of the response. (2) In the low activity state, the mean pitch amplitudes were relatively small compared with the noise generated by our image acquisition equipment. To assure that the SNR for the low activity state responses was not underestimated because of an overestimation of the noise, we calculated the SNR on the basis of third-order polynomial functions fitted to each individual response separately, approximating the time course of the responses without high-frequency noise. Irrespective of these details of data processing, we arrived at the same conclusion; that the SNR is not smaller in the high activity state than in the low activity state, despite the higher variability.

How might the optomotor gain switch be accomplished?

Halteres have been proposed to elevate the gain of head movements when they oscillate (Gilbert and Bauer, 1998; Huston, 2005; Sandeman, 1980). The large head jitter going along with high optomotor gain responses could be the consequence of a gain-modulating signal provided by the halteres. To clarify the role of the halteres as a possible cause of head jitter and high gain optomotor head pitch responses, we quantified the concurrency of head jitter and haltere movements. We found that in many trials the head jitter occurred with the same frequency as the oscillation of the observed haltere (Fig. 9). However, the power spectra of head movements and haltere oscillations did not in all trials display a distinct peak at the same frequency. This inconsistency may be due to the other (not observed) haltere possibly beating at another frequency, as sometimes happens (R.R., personal observation). Alternatively, peaks may be less distinct and not overlapping because of an occasional lack of a tight coupling between haltere oscillation and head jitter (see below).

For an evaluation of the concurrency of haltere movements and head jitter, we filmed four flies ventrally. The information about both halteres was important, because sometimes only one haltere was oscillating (data not shown). From the ventral view, we could determine when halteres oscillated but not their oscillation frequency because filming the basically up-and-down beating halteres from below leads to a motion signal at twice the oscillation frequency of the haltere. The haltere oscillation frequency can exceed 150 Hz, rendering the camera frame rate of 500 Hz insufficient to capture such a high-frequency signal.

To rule out that head jitter results from mechanical vibrations caused by thorax vibrations mediated by the activity of large power muscles potential thorax movements were scrutinised. Thorax movements, if existing at all, were too small to be resolved with our technical equipment. They were not distinguishable from those of a rigid object and, most importantly, were the same in the low and high activity states (Fig. 10A,B). By contrast, we observed vibrations of the thorax, when we did not fixate the wings, demonstrating that we could detect thorax vibrations with our experimental setup (data not shown). Hence, head jitter is probably not the consequence of mechanical vibrations of the whole fly but is the result of a signal coinciding with haltere oscillation. These findings are compatible with the hypothesis that the oscillating halteres provide a gating signal that sets the gain for the visually induced pitch movements and cause the head to jitter.

To further assess the relationship between haltere movements and head jitter, we quantified the coincidence of both movements for all trials in the time domain. For this quantification, we first analysed whether the halteres oscillated and whether the head jittered or not (for details see Materials and methods). Again, time bins of 20 ms

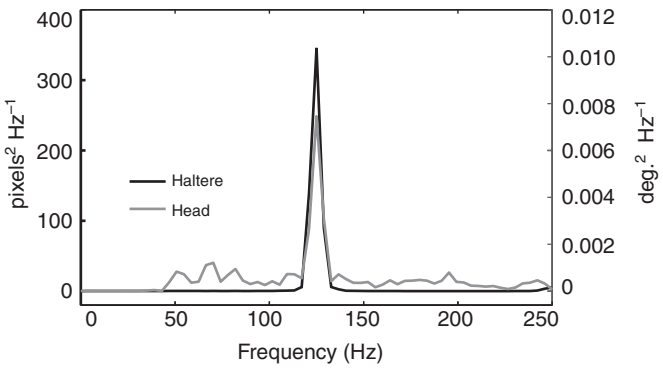

Fig. 9. Power spectra of haltere oscillation and head jitter. Power spectrum of a 50 Hz high-pass filtered head orientation trace (grey, right ordinate) and of haltere oscillations (black, left ordinate). The fly was filmed from the side to resolve the haltere oscillation frequency. The peak head jitter frequency of about 125 Hz corresponds well with the haltere oscillation frequency.

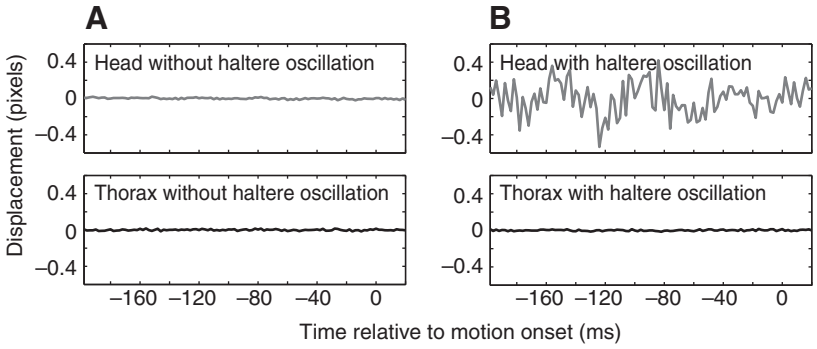

Fig. 10. Head pitch and thorax movements in the two activity states (high and low). The fly was filmed ventrally. (A) When the halteres did not oscillate, neither the head position (upper trace) nor the thorax position (lower trace) fluctuated with amplitudes resolvable by our technical equipment. (B) When the halteres oscillated, the head (upper trace) showed peculiar head jitter but the thorax (lower trace) did not.

were chosen because they are large enough to capture the oscillating activity of haltere and head movements and still short enough to provide information about the concurrency of head and haltere movements on a fine timescale. The histograms in Fig. 11 show, for one ventrally filmed fly, the relative occurrence of the strength of haltere movements for one haltere (Fig. 11A) and the strength of high-frequency head jitter (above 90 Hz) (Fig. 11B). Both histograms display two peaks representing no haltere oscillation and strong haltere oscillation in the one case and no head jitter and strong head jitter in the other case. These two peaks allowed us to separate two different activity states of the halteres and the head by setting a threshold analogous to what we did above (Fig. 3).

Do head jitter and haltere oscillations always coincide on a 20 ms timescale? If haltere oscillations would directly cause the head jitter, coincident activity is to be expected. To test this hypothesis, we investigated whether bins with (or without) haltere oscillation coincide with bins with (or without) head jitter. In most of the bins, there was neither haltere oscillation nor head jitter (Fig. 12A). When either activity occurred, haltere oscillations were accompanied by head jitter in most cases (Fig. 12B). However, occasionally, bins occurred with either only haltere oscillations or only head jitter. This result does not qualitatively depend on the exact choice of the thresholds that separate the two activity states for haltere oscillations and head jitter. Head jitter without coincident haltere oscillation and haltere movements without head jitter occurred mostly when the halteres or the head switched from being active to being passive and *vice versa*. Two examples for such state transitions are shown in Fig. 13. Head jitter often starts (Fig. 13A) and stops (Fig. 13B) earlier than haltere oscillation does, indicating halteres to be neither necessary nor sufficient for head jitter.

In summary, haltere oscillation and head jitter often coincided on a 20 ms timescale but the temporal overlap of haltere oscillation and head jitter was not perfect, casting doubts on the hypothesis that haltere oscillations cause head jitter and high gain optomotor responses.

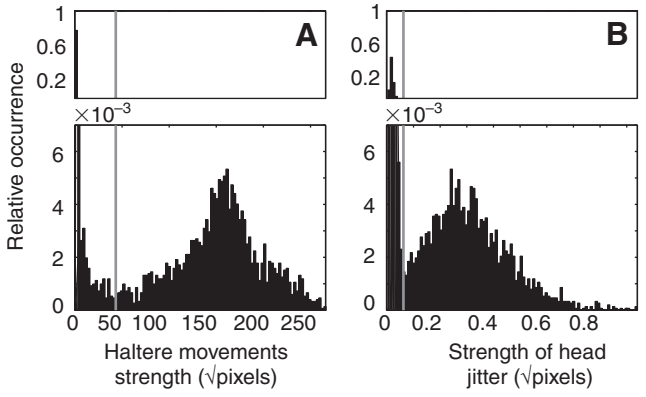

Fig. 11. Bimodal distributions of concurrently filmed high-frequency haltere movements and head jitter of one fly. (A) Frequency histogram of the strength of haltere movements for one of the two ventrally filmed halteres evaluated within 20 ms bins. All traces obtained from the fly were used for the histogram. The distribution of haltere movements is bimodal. Two activity states can be distinguished; large haltere oscillations (right peak) and small or no haltere oscillations (left peak). (B) Same as in A but for ventrally filmed head jitter movements. The bimodal distribution indicates the existence of two distinct activity states of the head with no head jitter and conspicuous head jitter, respectively. Between the two peaks, threshold values were set (grey vertical lines) for haltere movements and head jitter, respectively, to classify data according to the two activity states.

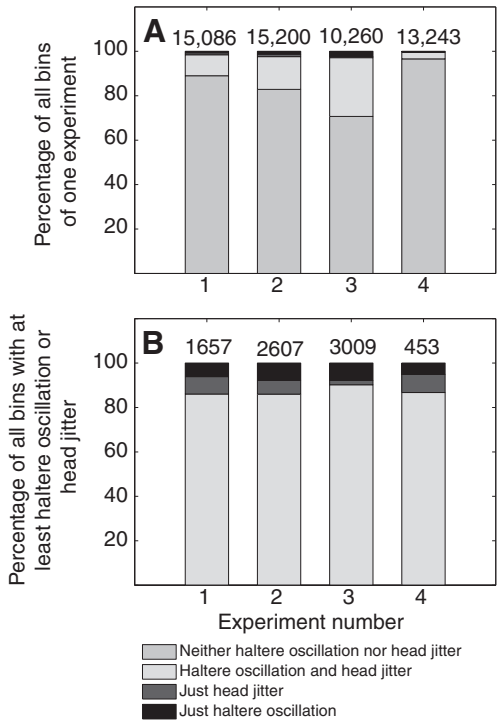

Fig. 12. Concurrency of head jitter and haltere movements. Percentages of 20 ms time bins with the four combinations of occurrence and non-occurrence of haltere oscillations and head jitter for four experiments on different flies. All bins in which at least one haltere oscillated were classified as 'haltere oscillation'. (A) Percentages of time bins relative to all bins of one experiment. In (B) these time bins were not taken into account during which neither halteres oscillated nor the head jittered. The total number of bins taken into account is shown on top of each column.

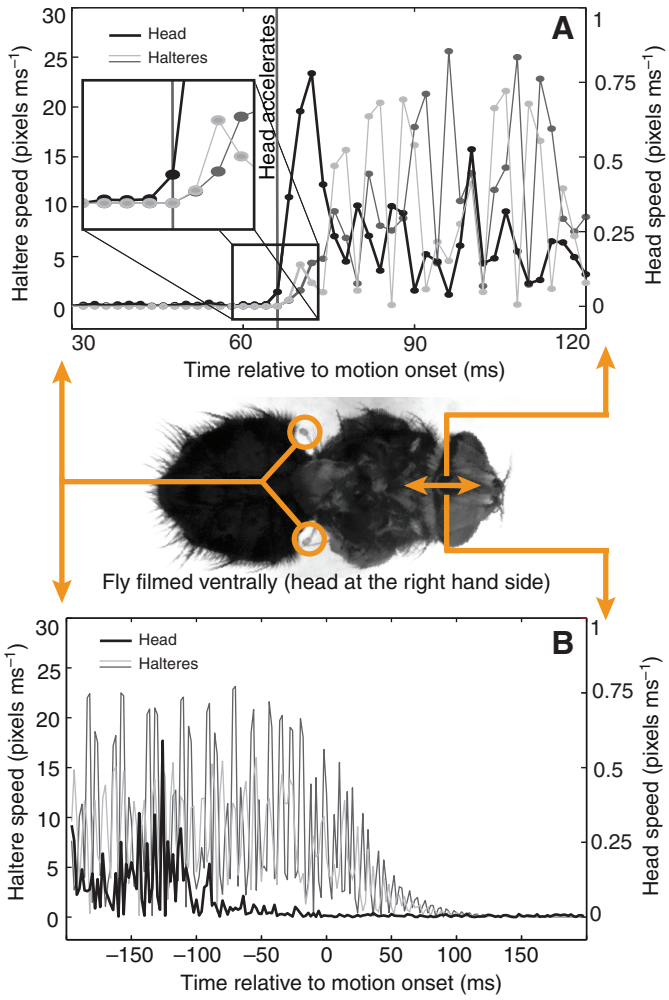

Fig. 13. Activity state transitions in a ventrally filmed fly. (A) Transition from low-to-high activity state within one trial. Speed of both halteres (grey lines, left ordinate) and head speed (black line, right ordinate) as a function of time. The head starts accelerating and thus undergoes a state transition before the halteres do (see also inset for finer temporal resolution). Therefore, haltere oscillations cannot account for the accelerated head movement. (B) Transition from high-to-low activity state. The head stops jittering before the halteres stop oscillating, indicating that haltere oscillation is not sufficient for head jitter.

How does the removal of the halteres affect head movements?

If haltere oscillations were responsible for head jitter and high optomotor gain, neither head jitter nor high gain optomotor responses should be observed after the ablation or immobilisation of the halteres. In three experiments on different flies, we removed the halteres by pulling them out of the thorax to assure that mechanoreceptor stimulation was no longer possible. In addition, in two further experiments, we immobilised the halteres with bees wax. We found that the visually induced head pitch responses before and after the removal of the halteres are similar (compare Fig. 14A, left and 14B, left). Even without halteres, large as well as small optomotor responses occur. Hence, our results clearly indicate the existence of a gain-modulating signal, which is not associated with reafferences signalling haltere movements. Whether the ablation of the halteres resulted in a slight reduction of the optomotor response gain in high gain responses cannot be decided due to the small sample size. Moreover, after haltere ablation, high-frequency fluctuations of the head were larger in trials with large optomotor responses than in trials with small optomotor responses (Fig. 14C), further indicating the action of a gain-modulating signal independent of haltere oscillation. Note, that the maximum jitter strength was reduced in all five experiments after ablation or immobilisation of the halteres (illustrated in Fig. 14A, right and 14B, right for one experiment). In the histogram showing the strength of head jitter as determined after high-pass filtering (see Materials and methods for details of analysing head jitter), only one pronounced peak (not two peaks) remains after haltere ablation (compare Fig. 14A, right and 14B, right). Thus, only with intact halteres is it possible to distinguish two activity states by evaluating head jitter movements. The finding of only one pronounced peak in the histogram (Fig. 14B, right) results from a change in the frequency content of the head jitter due to haltere removal. Such a change in the frequency content is corroborated by the fact that in the power spectra of the head position traces, as evaluated in Fig. 9, no distinct peak indicative of a rhythmicity of high-frequency head movements was observed after haltere ablation or immobilisation (data not shown).

In summary, our results show that the oscillating activity of the halteres does not cause large gain optomotor head pitch. Moreover, head jitter occurs when halteres are ablated and is therefore not exclusively caused by the halteres. Nevertheless haltere oscillations affect the strength of high-frequency head jitter.

DISCUSSION

From analysing the amount and source of variability in head optomotor pitch responses of tethered blowflies *Calliphora vicina* by high-speed cinematography, we obtained five main findings. (1) We identified two states of behavioural activity of the fly; one that is characterised by high optomotor gain responses accompanied by jittering movements of the head and the other by low optomotor gain and without obvious head jitter. We denote these two states as the high and low activity state, respectively. (2) The variability of the optomotor gain is larger in the high activity state than in the low activity state. (3) Despite the larger variability in the high activity state, the reliability of the optomotor pitch response as quantified by the SNR either remains constant across the two different gain states or may even be higher in the high activity state. (4) High gain optomotor head pitch responses are not the consequence of proprioceptive reafferences signalling whether halteres are oscillating. (5) The observed head jitter movements are, in part, caused by the halteres. In the following sections, we will first summarise what is known about the neuronal pathway mediating visually elicited head pitch responses. We will then discuss the observed behavioural variability and its sources as well as functional aspects of head optomotor pitch movements in the two activity states.

Neuronal control of optomotor head pitch movements

In order to mediate optomotor pitch responses of the head, the visual system has to provide the neck motor system with visual motion information. This information is provided by the compound eyes and conveyed by a subset of the ~50–60 large-field motion-sensitive visual inter-neurones in the third visual neuropile, the so-called tangential cells (TCs) (Huston and Krapp, 2008; Milde and Strausfeld, 1986; Milde et al., 1987; Strausfeld et al., 1987). Also, the ocelli were found to contribute at the level of TCs information about motion of the visual surroundings (Parsons et al., 2006). TCs are tuned to global optic flow elicited by different types of self-motion of the blowfly and are assumed to play a key role in processing visual motion information in the context of visually

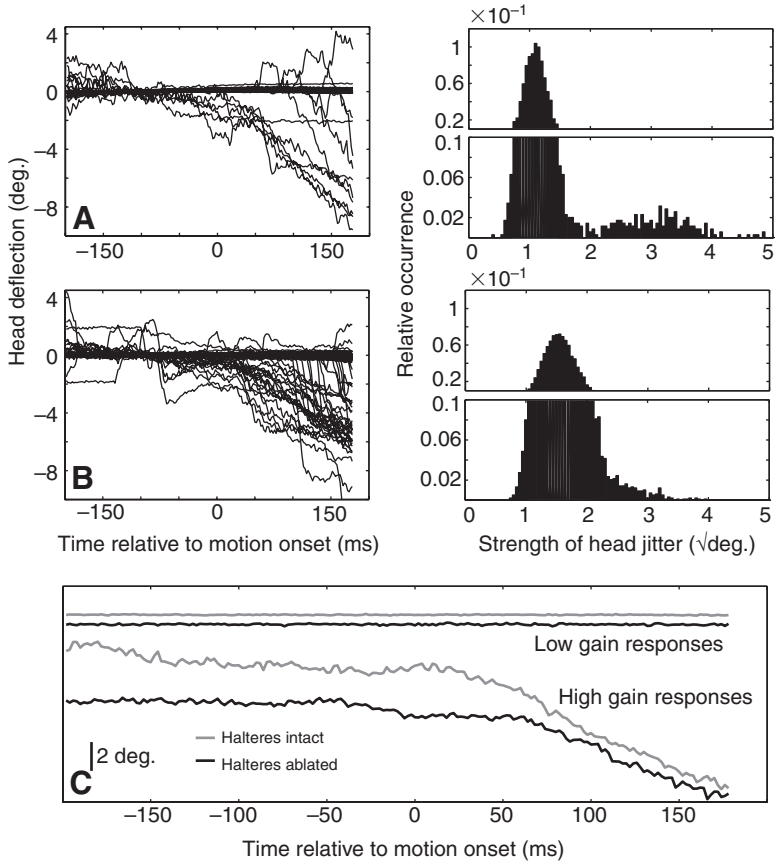

Fig. 14. Changes in head movements after ablation of both halteres. The fly was filmed laterally. Before (A) and after (B) ablation of both halteres. Left: the measured head orientation traces of all trials are displayed, irrespective of whether there was an activity state transition within a trial or not. These transitions explain why the separation into high and low activity state responses appears less clear than in Fig. 5. The traces were aligned to have zero mean in a 50 ms interval starting 42 ms before stimulus motion onset. Right: histograms of high-frequency head jitter strength. (C) Two example traces with high optomotor gain and two example traces with small optomotor gain. Both high gain traces, the one before (grey) and the one after (black) haltere ablation, show head jitter. But B (right) indicates a reduction in head jitter strength.

guided behaviour (Borst and Haag, 2002; Egelhaaf, 2006; Egelhaaf et al., 2005; Hausen and Egelhaaf, 1989; Krapp, 2000). Many TCs can be identified individually on the basis of their anatomical and physiological properties. For visually evoked head pitch movements, two TCs that are sensitive to downward motion in the frontal visual field, the so-called VS2 and VS3 cells (vertical system neurones), connect directly to motor neurones of the cervical nerve (CN), which in turn innervate muscles mediating pitch movements (Milde et al., 1987; Milde and Strausfeld, 1986; Strausfeld et al., 1987). Motor neurones in the frontal nerve (FN), which receive visual information from TCs *via* descending neurones and have been concluded to mediate head roll (Gilbert et al., 1995; Milde et al., 1987; Strausfeld et al., 1987), may also contribute to head pitch movements because electrical stimulation of the FN evokes head roll combined with a pitch component (Gilbert et al., 1995).

VS-cells, like other TCs, respond to the onset of a stimulus, as applied in the present study, with a sudden depolarisation of their membrane potential, reach their maximal response level after clearly less than 100 ms and then settle to a steady-state level after several hundreds of milliseconds to seconds (Egelhaaf and Borst, 1989; Grewe et al., 2006; Hengstenberg, 1982). By contrast, the stimulus-induced head pitch angle does not show a transient response peak but usually changes continually throughout the entire evaluation time of 178 ms as employed in our behavioural experiments (Fig. 6). Hence, the TC signal is integrated to transform a basically step-like neuronal response into a ramp-shaped pitch movement of the head. The integration of TC responses also takes place for the transformation of TC signals into optomotor yaw torque of the flying animal (Egelhaaf, 1987; Warzecha and Egelhaaf, 1996).

Variability of the head pitch optomotor gain

We found optomotor head pitch responses to be highly variable even though they were elicited by the identical visual stimulus. Moreover, other sensory input was not provided so that signals from non-visual modalities could only have changed due to internal fluctuations. Most of the variability we found in the responses is the consequence of the two alternative activity states that differ largely in their overall gain of the optomotor response. However, also within both activity states, the amplitudes of individual optomotor pitch responses differ greatly. What are the sources of the observed variability across (1) and within (2) the two activity states?

(1) We found head optomotor pitch movements to be either large or small, i.e. they occur in a bimodal manner. Obviously there is a signal that switches the gain of the optomotor responses. Halteres were suggested to provide a gain-modulating signal for fly head movements (Gilbert and Bauer, 1998; Huston, 2005; Sandeman, 1980). The halteres oscillate when the fly engages in locomotion (Sandeman and Markl, 1980) and their main function is to serve as equilibrium and gaze-stabilising organs by detecting Coriolis forces when the fly rotates in space (Dickinson, 1999; Nalbach, 1993; Nalbach, 1994; Nalbach and Hengstenberg, 1994; Pringle, 1948). This rotation detection is accomplished by the stimulation of mechanoreceptors at the base of the halteres (Chan and Dickinson, 1996; Pringle, 1948) when the halteres are deflected out of their swinging plane (Nalbach, 1994; Pringle, 1948; Sandeman, 1980; Sandeman and Markl, 1980). Also, the oscillations of the haltere themselves elicit action potentials in the haltere nerve phase-locked to the basic oscillation rhythm of the haltere (Fayyazuddin and Dickinson, 1996; Pringle, 1948). We propose that the reafferences that signal haltere oscillation are responsible for large head jitter

because the ablation or immobilisation of the halteres attenuates head jitter. Additionally, halteres and head often oscillate at the same frequency (Fig. 9). However, the reafferences are unlikely to account for the switch of the optomotor gain when the fly changes to the high activity state. This is particularly suggested by our findings, that even after the ablation or immobilisation of the halteres both, small as well as large optomotor pitch responses still occur (Fig. 14). Of course, the halteres could provide a complementary gain-modulating signal when the fly is already in the high activity state.

Could other reafferences provide the gain-modulating signals? Indeed, mechanoreceptors detecting movements of the wings or legs have been shown to modulate behavioural responses in different insect species [hawkmoth (Frye, 2001); locust (Steeves and Pearson, 1982)]. These mechanoreceptors are unlikely to play a gain-modulating role in our present experiments as neither the wings nor the legs were able to move during the experiments. Nonetheless receptors within the thorax may detect muscle activity if the fixated fly tries to fly or walk and could serve as reafferences. However, large muscle activity within the thorax is rather unlikely in our preparation because we did not observe noticeable thorax movements (Fig. 10), which can be expected to occur when the large power muscles are active. Therefore, we propose that a central signal directly elevates the gain of the optomotor response and, in parallel, induces the fly to walk or fly. Such a signal was described for the locust, modulating the transmission of sensory signals when the animal is flying (Reichert et al., 1985).

Irrespective of the origin of the gain-modulating signal, there are two principal mechanisms by which the modulation of the optomotor gain could be accomplished. (1) Either the visual signals are prevented to a large extent from inducing pitch movements during the low activity state or (2) the visual signals are boosted in the high activity state. For both of these mechanisms, there exists evidence from previous work. (1) In the flesh fly, *Neobellieria bullata*, the head is clamped to the thorax when flies are motionless; thus, preventing proprioceptive signals about head deflections from eliciting head movements (Gilbert and Bauer, 1998). This clamping is thought to be achieved by identified muscles that could pull the head to the trunk (Gilbert and Bauer, 1998; Strausfeld et al., 1987). Such a clamp, if it also exists in *Calliphora*, could be released by a gating signal when the fly switches from being stationary to walking or flying. (2) In *Calliphora*, electrophysiological evidence exists for an increase in the gain of head optomotor responses by gating the visual signals. Some of the motor neurones of the FN generate action potentials in response to visual stimulation only when the halteres are oscillating (Huston, 2005). As neck motor neurones in the FN mediate head roll (Gilbert et al., 1995; Milde et al., 1987; Strausfeld et al., 1987), the motor neurones that require haltere oscillation for their visual response are expected to mediate enhanced optomotor responses in roll direction when the halteres are active. If the motor neurones that control head pitch movements have similar properties with respect to a non-linear enhancement of the visual signal by a central gain-modulatory signal, they could account for the state dependence of the optomotor pitch response.

Where in the neuronal pathway could a gain-enhancing signal act? There are conflicting evidences with respect to state-dependent activity changes at the level of the TCs. On the one hand, even when explicitly addressing the possible effects of locomotor activity on the response properties of an identified TC, the so-called H1-neurone, no state-dependent differences in the responses were detected (Heide, 1983). On the other hand, activity changes have been reported to occur at the level of two other TCs (V1, V2) when applying octopamine that is associated with the arousal of insects (K. Longden, personal communication). However, it is rather unlikely that the response variability of TCs can account for the variability of head pitch responses across the activity states of the fly, because neither are responses bimodally distributed nor is response variability large enough (see below). This implies that the gain switch of the optomotor responses is expected to be mediated downstream of the visual system. As mentioned above the pathway for pitch head movements is very short; the visual motion information provided by TCs is directly transmitted to motor neurones of the CN that innervate the appropriate muscles in the neck. As already described, there is indeed electrophysiological evidence for gain modulation at the level of the motor neurones of the FN (Huston, 2005). However, in contrast to our conclusion of a central origin of the gain-modulating signal, Huston found that the gain depends on the oscillatory activity of the halteres (Huston, 2005). Two explanations may resolve this seeming discrepancy; either the visual signals are only gated by haltere signals in a subset of motor neurones and motor neurones mediating head pitch are not gated in this way or a central as well as a reafferent signal could jointly control the gain of optomotor head pitch responses. A central signal could be responsible for releasing the clamp pulling the head to the thorax, allowing the visual signals to evoke large head optomotor pitch, and the haltere reafference signals could boost visual signals further to get an even higher optomotor gain. This mechanism would account for our finding in that haltere oscillation is not necessary for large optomotor gain responses to occur but induces a haltere synchronous component of head jitter.

(2) What are the sources of the observed optomotor gain variability within a given activity state of the flies? All variability at the level of those TCs that mediate optomotor head movements should affect the variability of the behavioural response. The variability of TCs has been thoroughly characterised in the last two decades (Borst and Theunissen, 1999; Egelhaaf, 2006; Egelhaaf and Warzecha, 1999; Egelhaaf et al., 2005; Grewe et al., 2003; Karmeier et al., 2005; Lewen et al., 2001; Nemenman et al., 2008; Ruyter van Steveninck et al., 2001; Warzecha and Egelhaaf, 2001). Karmeier and colleagues (Karmeier et al., 2005) determined the response variability of a TC (VS1) with response properties very similar to VS2 and VS3. In the following text, we aim to estimate whether the noise at the level of the TCs is sufficient to explain the observed behavioural variability within a given activity state. The variability of the VS1-cell response depends on the membrane potential (Karmeier et al., 2005). We can assume that the response amplitude of VS-cells to a strong stimulus as used in the present study is in the range of 10–15 mV (Grewe et al., 2006). Under this assumption, the standard deviation can be expected to be in the range of 3.2–1.9, respectively (Karmeier et al., 2005). Taking the ratio of VS-cell response amplitude and the noise amplitude of VS1 approximated by the standard deviation, we arrive at a SNR of 3.1–7.9. This SNR is in good agreement with the SNR found by Warzecha and Egelhaaf (Warzecha and Egelhaaf, 1999) for a spiking TC under temperature conditions also applicable to our experiments. Temporal integration of the TCs response when being transformed into a head pitch response (see above) can be expected to further increase the SNR. Moreover, as more than one TC contributes to optomotor head pitch movements (Huston and Krapp, 2008; Milde et al., 1987; Milde and Strausfeld, 1986; Strausfeld et al., 1987) a further increase in the SNR is possible. However, the TC responses are largely correlated due to electrical coupling (Farrow et al., 2005; Haag and Borst, 2004) limiting the enhancement of the SNR. Although it is not possible without detailed model simulations to exactly quantify the consequences of all these processing steps for

the SNR, the variability found in TCs is probably too small to account for a SNR of 1.3–2.5 (Fig. 8) as we determined for head optomotor pitch responses. Hence, we suggest an additional noise source downstream of the TCs. This suggestion fits well to the finding that for modelling the optomotor yaw torque of tethered flying blowflies a considerable amount of noise had to be added to the response variability of TCs to account for the observed behavioural responses (Warzecha and Egelhaaf, 1996). We currently investigate the source of the variability that limits the behavioural performance in the sensory motor pathway responsible for head pitch movements by combining electrophysiological experiments and model simulations.

Functional aspects concerning the head pitch optomotor response

During free flight, blowflies turn in a discontinuous manner by making fast jerky rotations called saccades. In-between saccades, body and head orientation is kept almost constant (Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999). A similar saccadic gaze strategy was also found in walking blowflies (Blaj and van Hateren, 2004). In this way, the rotational components of image motion, including the pitch movements, are separated from the translational components. Only the translational optic flow component contains information about the 3-D layout of the environment, which was concluded to be represented by TCs (Karmeier et al., 2006; Kern et al., 2005; Kern et al., 2006). In the intersaccadic interval the stability of head orientation for roll, yaw and pitch is larger than that of the thorax (van Hateren and Schilstra, 1999). Hence, head optomotor responses can be expected to aid compensation for the residual rotational movements of the body. The receptive field organisation of TCs and neck motor neurones was shown to be particularly suited to encode the rotational components experienced by the fly when the head rotates in the environment (Huston and Krapp, 2008; Krapp et al., 1998). Moreover, there are several other sensory mechanisms that contribute to compensate rotatory body movements (Hengstenberg, 1991; Hengstenberg, 1993).

To what degree do head optomotor pitch responses, as characterised in the present study, contribute to gaze stabilisation? If the head velocity would equal the pattern velocity, the optomotor gain was 1, indicating a full compensation of the retinal slip. We determined the mean gain of the optomotor head pitch response in the high activity state to be only 0.2. In the low activity state, the optomotor gain was about one order of magnitude smaller (approximately 0.01). It is unlikely that head optomotor following responses in the low activity state are of any functional significance because of their minute amplitude. These pitch movements might be due to neuronal activity transmitted to the neck muscles resulting from incomplete suppressed visual signals (see above for a more detailed discussion of this point).

In the high activity state, the optomotor head pitch response compensates for about 20% of the retinal image slip, which is much less than the compensation of more than 60% observed for optomotor roll responses of the head (Hengstenberg, 1991; Hengstenberg, 1993; Stange and Hengstenberg, 1996). However, the compensation of the retinal image slip for 20%, which was determined in the present study is in agreement with another study of optomotor head pitch done with *Musca* (Kirschfeld, 1989). These differences may be explained by several reasons apart from a potential genuine difference in the gain of optomotor pitch and roll responses. (1) The discrepancies may result from methodological differences. In the above mentioned studies on roll movements of the head, the gain was determined for the steady state response to sinusoidally oscillating pattern velocity whereas we determined the gain of the head pitch system for the early response phase to a velocity step. Head optomotor response gain was found to depend largely on the temporal structure of the stimulus paradigm (Kirschfeld, 1989). (2) Potentially, the small gain of optomotor head pitch is due to a weaker visual stimulation than was used in the head roll experiments. The optomotor gain depends on the stimulus velocity in a non-linear manner (Hengstenberg, 1984; Warzecha and Egelhaaf, 1996). We chose the stimulus velocity of 168 deg. s^{-1} to elicit a large optomotor response amplitude minimising the influence of noise, resulting from our technical equipment when estimating the variability of the optomotor responses. Maximising the response amplitude does not necessarily maximise optomotor gain, i.e. the ratio of response amplitude and stimulus velocity. Indeed, our preliminary tests of the velocity dependence of the head pitch indicate that, in agreement with studies by Hengstenberg (Hengstenberg, 1991) on head roll, the optomotor gain would have been somewhat larger for lower stimulus velocities. However, this finding is unlikely to account for a more than a threefold difference in gain. Moreover, the spatial extent of the stimulus pattern may cause differences in optomotor gain. In particular, our stimulus only targets the frontal visual field whereas neck motor neurones potentially driving head movements were found to be sensitive to upward motion also in the caudo–lateral visual field (Huston, 2005). (3) In the studies on head roll, tethered flying flies were investigated in a wind tunnel whereas in our experiments, which were performed without air flow, the wings of the flies were removed or fixated. Possibly mechanoreceptors detecting wing movements, which have been shown to exist in the blowfly (Heide, 1983), or airflow (Taylor and Krapp, 2007) may increase the optomotor gain. Such a mechanism cannot be excluded because in the hawkmoth proprioceptive signals were shown to change the gain of optomotor body lift (Frye, 2001). (4) Optomotor pitch responses in the high activity state may be limited by a saturation due to a mechanical stop of the head. This possibility is rather unlikely because the time course of individual head pitch responses is similar for responses close to and distant from the lowest pitch orientation observed (not shown). (5) It is also rather unlikely that the low optomotor gain of pitch responses is caused by an upper limit in head velocity given by the neck motor machinery. The head pitch velocity of the blowfly can assume values of considerably more than 168 deg. s^{-1}, the velocity of our stimulus [see transition from low to high activity state in Fig. 4 and Nalbach and Hengstenberg (Nalbach and Hengstenberg, 1994)].

As touched on above, the amplitude of compensatory head responses is not exclusively set by the gain of the optomotor response mediated by the compound eyes. Instead, several other sensory systems contribute to head stabilisation (Hengstenberg, 1991; Hengstenberg, 1993; Krapp and Wicklein, 2008; Taylor and Krapp, 2007). In flying flies, also the mechanosensory equilibrium system, the halteres sense rotations of the animal and cause steering manoeuvres as well as head movements (Hengstenberg, 1988; Nalbach, 1993; Nalbach, 1994; Nalbach and Hengstenberg, 1994) compensating for an unwanted image motion on the retina. Tethered flying flies compensate with their head for about 80% of the angular velocity detected by their halteres during pitch rotations (Nalbach and Hengstenberg, 1994), and there is good evidence for summation of visual and haltere feedback during body rotations in *Drosophila* (Sherman and Dickinson, 2004) and for head roll in *Calliphora* (Hengstenberg, 1993). Moreover, ocellar stimulation contributes to compensatory head movements (Hengstenberg, 1991; Hengstenberg,

1993) and modifies the activity of the VS-cells (Parsons et al., 2006). When the fly moves around, the overall gain of pitch movements may therefore well be in a functionally meaningful range.

What might be the functional significance of two different optomotor gain states? Stabilising the head orientation between saccadic turns is only meaningful during locomotion (see above). This suggests that the periods of time when the gain of the optomotor system is large is confined to periods when flies walk or fly under natural conditions. This is corroborated by the findings of other studies that, in blowflies, head movements elicited by sensory input are larger when the flies walk or fly (Gilbert and Bauer, 1998; Hengstenberg et al., 1986; Horn and Lang, 1978). In particular, we found head jitter to go along with large optomotor responses as well as with the oscillation of the halteres, implying that large optomotor responses occur when the fly walks or flies. This association arises because flies are known to oscillate their halteres when they locomote (Sandeman and Markl, 1980). However, when the fly is immobile, it is not necessary to have a high optomotor gain because there is no need to stabilise the head. Moreover, a high gain may even be disadvantageous because the higher gain goes along with a considerable head jitter. To detect movements in the surroundings, such as an approaching predator, might be easier when the head moves as little as possible. Last but not least energy constraints (Laughlin, 2001) may favour the existence of two activity states.

A closer look at the source and functional significance of head jitter movements

Head jitter often occurred with the same frequency as haltere oscillation (Fig. 9). Additionally the overall amplitude of head jitter was reduced when the halteres were removed (Fig. 14). In principle, two sources of head jitter are conceivable; a mechanical one and a neuronal one.

Van Hateren and Schilstra also found and analysed head jitter in flies and suggested a mechanical source (Van Hateren and Schilstra, 1999). However, the head jitter they observed occurred during free flight and is probably due to thorax oscillations produced by the wing beat. Because we removed the wings and the legs and fixated the stumps, it is not possible that the head jitter we observed was caused by mechanical vibrations elicited by wing or leg movements. Furthermore, in our preparation with removed wings and legs, we did not observe any obvious vibrations of the thorax, even when the head was jittering and the halteres were oscillating (Fig. 10). By contrast, we observed vibrations of the thorax, when we did not fixate the wings, demonstrating that we could detect thorax vibrations with our experimental setup. Therefore we exclude a mechanical source and propose a neuronal mechanism causing head jitter associated with haltere movements. The head jitter is probably either (1) the inevitable consequence of the halteres function to mediate gaze-stabilising head movements or (2) is due to an additional optomotor gain-modulating signal provided by the halteres.

(1) To meet their main functions as equilibrium and gaze-stabilising organs, halteres detect Coriolis forces when the fly rotates in space (Dickinson, 1999; Nalbach, 1993; Nalbach, 1994; Nalbach and Hengstenberg, 1994; Pringle, 1948). This detection is accomplished by the stimulation of mechanoreceptors at the base of the halteres (Chan and Dickinson, 1996; Pringle, 1948) when the halteres are deflected out of their swinging plane (Nalbach, 1994; Pringle, 1948; Sandeman, 1980; Sandeman and Markl, 1980). Also, the oscillations of the halteres themselves elicit action potentials in the haltere nerves phase-locked to the basic oscillation rhythm of the haltere (Fayyazuddin and Dickinson, 1996; Pringle, 1948). These action potentials could be modulated in their phase or number of occurrence to mediate Coriolis force-induced head movements. It is well conceivable that the phase-locked action potentials are responsible for the observed head jitter requiring that the temporal occurrence of transient membrane potential deflections is maintained up to the level of the neck muscles. In fact, at the level of the motor neurones supplying the neck and steering muscles such a temporal precision has been characterised (Huston, 2005; Fayyazuddin and Dickinson, 1996). As haltere-mediated head stabilisation is accomplished on a timescale of only few milliseconds (Nalbach and Hengstenberg, 1994), it is not advisable from a functional point of view to smooth out the transient phase-locked signals by temporal integration. Instead, the dynamic properties of the neck muscles are adjusted to mediate very fast head movements when the halteres detect rotations of the fly. The system may well cope with the small high-frequency head jitter as an inevitable consequence of the mechanism detecting haltere signals because head jitter is unlikely to disturb visual scene analysis considerably due to its small amplitude and high frequency.

(2) As already stressed above, an alternative or additional function of the haltere beat-synchronous signals could be the elevation of the gain of visually induced head movements when the fly walks or flies. At least in the case of head optomotor pitch, this would however, at the most, be an additional source of gain modulation (see above). This explanation for a neuronal mechanism mediating head jitter and gain modulation is analogous to what has already been proposed for a flight steering muscle of the fly. At this level, action potentials occur only when wing beat-synchronous afferent signals and visual signals are present simultaneously, indicating the presence of a gating or gain-modulating mechanism (Heide, 1975; Heide, 1983). Another interesting analogy between the neck and steering muscle activity besides the gain-modulating mechanism is the jitter observed in an identified steering muscle reminiscent of head jitter when electrically stimulating the motor nerve with a frequency mimicking the wing beat frequency (Heide, 1971; Heide, 1983).

On the basis of these proposed explanations for the head jitter, one would expect the head jitter to always occur phase-locked to the haltere beat frequency. However, this was not the case. Properties of neck motor neurones as described for those of the FN (Huston, 2005) may explain this apparent discrepancy. According to Huston (Huston, 2005), the action potential frequency found in neck motor neurones of the FN sometimes: (1) equals the haltere oscillation frequency even without applying a visual stimulus, (2) reflects the haltere beat frequency only when a visual stimulus is applied simultaneously and (3) may even be higher than the haltere beat frequency because of eliciting more than just one action potential in one cycle. Moreover, the two halteres of a fly sometimes may oscillate with different frequencies. Because we only determined the oscillation frequency of the filmed haltere, the head jitter may be not recognised as being phase-locked to two different oscillation frequencies. It may be expected that, in contrast to our observation, head jitter does not occur when the halteres are ablated. However, transient signals impinging on the neck motor system, such as action potentials evoked by visual stimulation, also provide high-frequency input possibly leading to head jitter.

In conclusion, we found that the head optomotor pitch responses of blowflies depend on the animal's internal state, being either large (high activity state) or small (low activity state). Even within a given state the optomotor gain is variable. This variability is

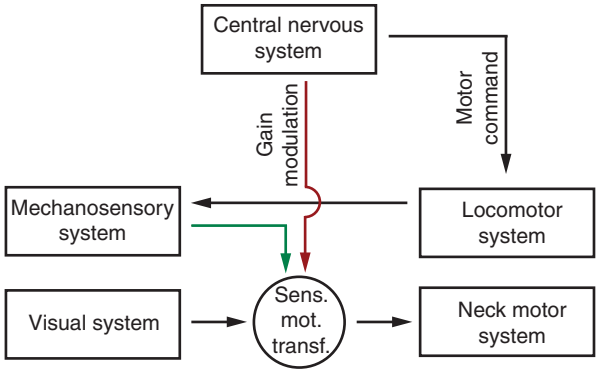

Fig. 15. Diagram illustrating our current hypothesis about the mechanisms that modify the gain of visually induced head pitch and the origin of the head jitter. A motor command from the central nervous system initiates the fly to walk or fly. Additionally the central nervous system elevates the gain of head optomotor responses (red arrow). Movements associated with locomotor behaviour, namely haltere oscillations, are sensed by the mechanosensory system and cause head jitter movements (green arrow). On the basis of our experiments, it remains open whether the signal from the halteres contributes to modifying the gain of optomotor head pitch responses. Note, that this diagram is not meant to capture the entire complexity of the sensory motor interface controlling head pitch movements. It only shows the relation between those parts of the system that are the focus of the present study. Sens. mot. trans., sensory-motor transformation.

higher in the high activity state than in the low activity state. The optomotor gain switch is not provided by reafferences originating at mechanoreceptors detecting haltere movements. We conclude that a central signal going along with initiating and maintaining locomotor activity of the fly is the most plausible source of gain control (Fig. 15). Nonetheless, the transient input to the head pitch control system phase-locked to haltere frequency as reflected in the observed head jitter could, in accordance with previous studies (Huston, 2005), provide an additional gain-modulating signal. Alternatively, head jitter may be the inevitable consequence of the dynamic properties of the neck muscles designed to mediate very fast head movements when the halteres detect rotations of the fly.

LIST OF ABBREVIATIONS

CN	cervical nerve
FN	frontal nerve
ROI	region of interest
SNR	signal-to-noise ratio
TC	tangential cell
VS1, VS2, VS3	vertical system neurones 1, 2 and 3, respectively

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REFERENCES

Blaj, G. and van Hateren, J. H. (2004). Saccadic head and thorax movements in freely walking blowflies. *J. Comp. Physiol. A* **190**, 861-868.
Borst, A. and Haag, J. (2002). Neural networks in the cockpit of the fly. *J. Comp. Physiol. A* **188**, 419-437.
Borst, A. and Theunissen, F. E. (1999). Information theory and neural coding. *Nat. Neurosci.* **2**, 947-957.
Chan, W. P. and Dickinson, M. H. (1996). Position-specific central projections of mechanosensory neurons on the haltere of the blow fly, *Calliphora vicina*. *J. Comp. Neurol.* **369**, 405-418.
Dickinson, M. H. (1999). Haltere-mediated equilibrium reflexes of the fruit fly, *Drosophila melanogaster*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **354**, 903-916.
Egelhaaf, M. (1985). On the neuronal basis of figure-ground discrimination by relative motion in the visual system of the fly. II. Figure-detection cells, a new class of visual interneurones. *Biol. Cybern.* **52**, 195-209.
Egelhaaf, M. (1987). Dynamic properties of two control systems underlying visually guided turning in house-flies. *J. Comp. Physiol. A* **161**, 777-783.
Egelhaaf, M. (2006). The neural computation of visual motion. In *Invertebrate Vision* (ed. E. Warrant and D. E. Nilsson). Cambridge: Cambridge University Press.
Egelhaaf, M. and Borst, A. (1989). Transient and steady-state response properties of movement detectors. *J. Opt. Soc. Am. A* **6**, 116-127.
Egelhaaf, M. and Borst, A. (1993). Motion computation and visual orientation in flies. *Comp. Biochem. Physiol.* **104A**, 659-673.
Egelhaaf, M. and Warzecha, A.-K. (1999). Encoding of motion in real time by the fly visual system. *Curr. Opin. Neurobiol.* **9**, 454-460.
Egelhaaf, M., Grewe, J., Karmeier, K., Kern, R., Kurtz, R. and Warzecha, A. K. (2005). Novel approaches to visual information processing in insects: case studies on neuronal computations in the blowfly. In *Methods in Insect Sensory Neuroscience* (ed. T. A. Christensen), pp. 185-212. Boca Raton, FL: CRC Press.
Faisal, A. A., Selen, L. P. and Wolpert, D. M. (2008). Noise in the nervous system. *Nat. Rev. Neurosci.* **9**, 292-303.
Farrow, K., Borst, A. and Haag, J. (2005). Sharing receptive fields with your neighbors: tuning the vertical system cells to wide field motion. *J. Neurosci.* **25**, 3985-3993.
Fayyazuddin, A. and Dickinson, M. H. (1996). Haltere afferents provide direct, electrotonic input to a steering motor neuron in the blowfly, *Calliphora*. *J. Neurosci.* **16**, 5225-5232.
Frye, M. A. (2001). Effects of stretch receptor ablation on the optomotor control of lift in the hawkmoth *Manduca sexta*. *J. Exp. Biol.* **204**, 3683-3691.
Frye, M. A. and Dickinson, M. H. (2001). Fly flight: a model for the neural control of complex behavior. *Neuron* **32**, 385-388.
Gilbert, C. and Bauer, E. (1998). Resistance reflex that maintains upright head posture in the flesh fly *Neobellieria bullata* (Sarcophagidae). *J. Exp. Biol.* **201**, 2735-2744.
Gilbert, C., Gronenberg, W. and Strausfeld, N. J. (1995). Oculomotor control in calliphorid flies: head movements during activation and inhibition of neck motor-neurons corroborate neuroanatomical predictions. *J. Comp. Neurol.* **361**, 285-297.
Gnatzy, W., Grunert, U. and Bender, M. (1987). Campaniform sensilla of calliphora-vicina (Insecta, Diptera).1. Topography. *Zoomorphology* **106**, 312-319.
Grewe, J., Kretzberg, J., Warzecha, A. K. and Egelhaaf, M. (2003). Impact of photon-noise on the reliability of a motion-sensitive neuron in the fly's visual system. *J. Neurosci.* **23**, 10776-10783.
Grewe, J., Matos, N., Egelhaaf, M. and Warzecha, A. K. (2006). Implications of functionally different synaptic inputs for neuronal gain and computational properties of fly visual interneurons. *J. Neurophysiol.* **96**, 1838-1847.
Grewe, J., Weckström, M., Egelhaaf, M. and Warzecha, A. K. (2007). Information and discriminability as measures of reliability of sensory coding. *PloS one* **2**, e1328.
Haag, J. and Borst, A. (1997). Encoding of visual motion information and reliability in spiking and graded potential neurons. *J. Neurosci.* **17**, 4809-4819.
Haag, J. and Borst, A. (2004). Neural mechanism underlying complex receptive field properties of motion-sensitive interneurons. *Nat. Neurosci.* **7**, 628-634.
Hardie, R. C. (1979). Electrophysiological analysis of fly retina. I: Comparative properties of R1-6 and R 7 and 8. *J. Comp. Physiol. A* **129**, 19-33.
Hausen, K. and Egelhaaf, M. (1989). Neural mechanisms of visual course control in insects. In *Facets of Vision* (ed. D. Stavenga and R. C. Hardie), pp. 391-424. Berlin: Springer.
Heide, G. (1971). Die Funktion der nicht-fibrillären Flugmuskeln von Calliphora: Teil II Muskuläre Mechanismen der Flugsteuerung und ihre nervöse Kontrolle. *Zool. Jb. Physiol.* **76**, 99-137.
Heide, G. (1975). Properties of a motor output system involved in the optomotor response in flies. *Biol. Cybern.* **20**, 99-112.
Heide, G. (1983). Neural mechanisms of flight control in Diptera. In *BIONA Report* (ed. W. Nachtigall), pp. 35-52. Mainz: Akademie der Wissenschaften und der Literatur zu Mainz and Gustav Fischer Verlag.
Hengstenberg, R. (1982). Common visual response properties of giant vertical cells in the lobula plate of the blowfly *Calliphora*. *J. Comp. Physiol.* **149**, 179-193.
Hengstenberg, R. (1984). Roll-stabilization during flight of the blowfly's head and body by mechanical and visual cues. In *Localization and Orientation in Biology and Engineering* (ed. D. Varju and H. Schnitzler), pp. 121-134. New York: Springer.
Hengstenberg, R. (1988). Mechanosensory control of compensatory head roll during flight in the blowfly *Calliphora erythrocephala* Meig. *J. Comp. Physiol. A* **163**, 151-165.
Hengstenberg, R. (1991). Gaze control in the blowfly *Calliphora*: a multisensory, two-stage integration process. *Semin. Neurosci.* **3**, 19-29.
Hengstenberg, R. (1993). Multisensory control in insect oculomotor systems. In *Visual Motion and its Role in the Stabilization of Gaze* (ed. F. A. Miles and J. Wallman), pp. 285-298. New York: Elsevier.
Hengstenberg, R., Sandeman, D. C. and Hengstenberg, B. (1986). Compensatory head roll in the blowfly *Calliphora* during flight. *Proc. R. Soc. Lond. B* **227**, 455-482.
Horn, E. and Lang, H. G. (1978). Positional head reflexes and role of prosternal organ in walking fly, *Calliphora erythrocephala*. *J. Comp. Physiol.* **126**, 137-146.
Huston, S. (2005). Neural basis of a visuo-motor transformation in the fly. PhD thesis. Cambridge University, Cambridge, UK.
Huston, S. J. and Krapp, H. G. (2008). Visuomotor transformation in the fly gaze stabilization system. *Plos Biol.* **6**, 1468-1478.
Johnston, D. and Wu, M. S. (1995). *Foundations of Cellular Neurophysiology*. Cambridge, MA: MIT Press.
Juusola, M., Kouvalainen, E., Järvilehto, M. and Weckström, M. (1994). Contrast gain, signal-to-noise ratio and linearity in light-adapted blowfly photoreceptors. *J. Gen. Physiol.* **104**, 593-621.

Karmeier, K., Krapp, H. G. and Egelhaaf, M. (2005). Population coding of self-motion: applying Bayesian analysis to a population of visual interneurons in the fly. *J. Neurophysiol.* **94**, 2182-2194.
Karmeier, K., van Hateren, J. H., Kern, R. and Egelhaaf, M. (2006). Encoding of naturalistic optic flow by a population of blowfly motion sensitive neurons. *J. Neurophysiol.* **96**, 1602-1614.
Kern, R., van Hateren, J. H., Michaelis, C., Lindemann, J. P. and Egelhaaf, M. (2005). Function of a fly motion-sensitive neuron matches eye movements during free flight. *PLOS Biol.* **3**, 1130-1138.
Kern, R., van Hateren, J. H. and Egelhaaf, M. (2006). Representation of behaviourally relevant information by blowfly motion-sensitive visual interneurons requires precise compensatory head movements. *J. Exp. Biol.* **209**, 1251-1260.
Kirschfeld, K. (1989). Automatic gain control in movement detection of the fly. *Naturwissenschaften* **76**, 378-380.
Krapp, H. G. (2000). Neuronal matched filters for optic flow processing in flying insects. *Int. Rev. Neurobiol.* **44**, 93-120.
Krapp, H. G. and Wicklein, M. (2008). Central processing of visual information in insects. In *The Senses: A Comprehensive Reference* Vol. 1, Vision I (ed. Allan I. Basbaum, Akimichi Kaneko, Gordon M. Shepherd and Gerald Westheimer), pp. 131-204. Oxford: Elsevier.
Krapp, H. G., Hengstenberg, B. and Hengstenberg, R. (1998). Dendritic structure and receptive-field organization of optic flow processing interneurons in the fly. *J. Neurophysiol.* **79**, 1902-1917.
Laughlin, S. B. (2001). Energy as a constraint on the coding and processing of sensory information. *Curr. Opin. Neurobiol.* **11**, 475-480.
Lewen, G. D., Bialek, W. and Ruyter van Steveninck, R. d. (2001). Neural coding of naturalistic motion stimuli. *Network* **12**, 317-329.
Milde, J. J. and Strausfeld, N. J. (1986). Visuo-motor pathways in arthropods: giant motion-sensitive neurons connect compound eyes directly to neck muscles in blowflies (Calliphora-Erythrocephala). *Naturwissenschaften* **73**, 151-154.
Milde, J. J., Seyan, H. S. and Strausfeld, N. J. (1987). The neck motor system of the fly calliphora-erythrocephala. 2. Sensory organization. *J. Comp. Physiol. A* **160**, 225-238.
Nalbach, G. (1993). The halteres of the blowfly *Calliphora:* I. Kinematics and dynamics. *J. Comp. Physiol. A* **173**, 293-300.
Nalbach, G. (1994). Extremely nonorthogonal axes in a sense organ for rotation: behavioral-analysis of the dipteran haltere system. *Neuroscience* **61**, 149-163.
Nalbach, G. and 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.
Nemenman, I., Lewen, G. D., Bialek, W. and de Ruyter van Steveninck, R. R. (2008). Neural coding of natural stimuli: information at sub-millisecond resolution. *PLoS Comput. Biol.* **4**, e1000025.
Nolen, T. G. and Hoy, R. R. (1984). Initiation of behavior by single neurons: the role of behavioral context. *Science* **226**, 992-994.
Parsons, M. M., Krapp, H. G., Laughlin, S. B. (2006). A motion-sensitive neurone responds to signals from the two visual systems of the blowfly, the compound eyes and ocelli. *J. Exp. Biol.* **209**, 4464-4474.
Poulet, J. F. A. and Hedwig, B. (2005). Auditory orientation in crickets: pattern recognition controls reactive steering. *Proc. Natl. Acad. Sci. USA* **102**, 15665-15669.
Pringle, J. W. S. (1948). The gyroscopic mechanism of the halteres of Diptera. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **233**, 347-384.
Reichert, H., Rowell, C. H. F. and Griss, C. (1985). Course correction circuitry translates feature detection into behavioral action in locusts. *Nature* **315**, 142-144.
Rodieck, R. W. (1998). *The First Steps in Seeing.* Sunderland, MA: Sinauer.
Ruyter van Steveninck, R. D. and Laughlin, S. B. (1996). The rate of information transfer at graded-potential synapses. *Nature* **379**, 642-645.
Ruyter van Steveninck, R. d., Borst, A. and Bialek, W. (2001). Real-time encoding of motion: answerable questions and questionable answers from the fly's visual system. In *Motion Vision* (ed. J. M. Zanker and J. Zeil), pp. 279-306. New York: Springer.
Sandeman, D. C. (1980). Angular-acceleration, compensatory head movements and the halteres of flies (Lucilia-Serricata). *J. Comp. Physiol.* **136**, 361-367.
Sandeman, D. C. and Markl, H. (1980). Head movements in flies (Calliphora) produced by deflection of the halteres. *J. Exp. Biol.* **85**, 43-60.
Schilstra, C. and van Hateren, J. H. (1998). Stabilizing gaze in flying blowflies. *Nature* **395**, 654.
Schilstra, C. and van Hateren, J. H. (1999). Blowfly flight and optic flow. I. Thorax kinematics and flight dynamics. *J. Exp. Biol.* **202**, 1481-1490.
Sherman, A. and Dickinson, M. H. (2004). Summation of visual and mechanosensory feedback in *Drosophila* flight control. *J. Exp. Biol.* **207**, 133-142.
Sillar, K. T. and Roberts, A. (1988). A neuronal mechanism for sensory gating during locomotion in a vertebrate. *Nature* **331**, 262-265.
Stange, G. and Hengstenberg, R. (1996). Tri-axial, real-time logging of fly head movements. *J. Neurosci. Methods* **64**, 209-218.
Staudacher, E. and Schildberger, K. (1998). Gating of sensory responses of descending brain neurones during walking in crickets. *J. Exp. Biol.* **201**, 559-572.
Steeves, J. D. and Pearson, K. G. (1982). Proprioceptive gating of inhibitory pathways to hindleg flexor moto-neurons in the locust. *J. Comp. Physiol.* **146**, 507-515.
Strausfeld, N. J., Seyan, H. S. and Milde, J. J. (1987). The neck motor system of the fly calliphora-erythrocephala. 1. Muscles and motor neurons. *J. Comp. Physiol. A* **160**, 205-224.
Taylor, G. K. and Krapp, H. G. (2007). Sensory systems and flight stability: what do insects measure and why? *Adv. Insect Physiol.* **34**, 231-316.
van Hateren, J. H. and Schilstra, C. (1999). Blowfly flight and optic flow. II. Head movements during flight. *J. Exp. Biol.* **202**, 1491-1500.
Warzecha, A. K. and Egelhaaf, M. (1996). Intrinsic properties of biological motion detectors prevent the optomotor control system from getting unstable. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **351**, 1579-1591.
Warzecha, A. K. and Egelhaaf, M. (1999). Variability in spike trains during constant and dynamic stimulation. *Science* **283**, 1927-1930.
Warzecha, A. K. and Egelhaaf, M. (2001). Neuronal encoding of visual motion in real-time. In *Processing Visual Motion in the Real World: A Survey of Computational, Neural, and Ecological Constraints* (ed. J. M. Zanker and J. Zeil), pp. 239-277. New York: Springer.
Warzecha, A. K., Kretzberg, J. and Egelhaaf, M. (1998). Temporal precision of the encoding of motion information by visual interneurons. *Curr. Biol.* **8**, 359-368.
Warzecha, A. K., Kretzberg, J. and Egelhaaf, M. (2000). Reliability of a fly motion-sensitive neuron depends on stimulus parameters. *J. Neurosci.* **20**, 8886-8896.
Zanker, J. M., Egelhaaf, M. and Warzecha, A. K. (1991). On the coordination of motor output during visual flight control of flies. *J. Comp. Physiol. A* **169**, 127-134.