The Journal of Experimental Biology 213, 331-338
Published by The Company of Biologists 2010
doi:10.1242/jeb.035386

Behavioural state affects motion-sensitive neurones in the fly visual system

R. Rosner*, M. Egelhaaf and A.-K. Warzecha

Lehrstuhl für Neurobiologie, Universität Bielefeld, Bielefeld, Germany

*Author for correspondence at present address: Fachbereich Biologie, Tierphysiologie, Philipps-Universität Marburg, Marburg, Germany (rosner@staff.uni-marburg.de)

Accepted 5 October 2009

SUMMARY

The strength of stimulus-induced responses at the neuronal and the behavioural level often depends on the internal state of an animal. Within pathways processing sensory information and eventually controlling behavioural responses, such gain changes can originate at several sites. Using motion-sensitive lobula plate tangential cells (LPTCs) of blowflies, we address whether and in which way information processing changes for two different states of motor activity. We distinguish between the two states on the basis of haltere movements. Halteres are the evolutionarily transformed hindwings of flies. They oscillate when the animals walk or fly. LPTCs mediate, amongst other behaviours, head optomotor responses. These are either of large or small amplitude depending on the state of motor activity. Here we find that LPTC responses also depend on the motor activity of flies. In particular, LPTC responses are enhanced when halteres oscillate. Nevertheless, the response changes of LPTCs do not account for the corresponding large gain changes of head movements. Moreover, haltere activity itself does not change the activity of LPTCs. Instead, we propose that a central signal associated with motor activity changes the gain of head optomotor responses and the response properties of LPTCs.

Key words: LPTC, arousal, behavioural state, fly, motor activity, visual system.

INTRODUCTION

When an animal moves through the world the images on its retinae are in continuous motion. Processing visual motion endows the animal with information about its self-movement and the three-dimensional layout of its environment. The nervous systems of many mobile animals are able to evaluate this 'optic flow' and to exploit this information to mediate appropriate behavioural actions (Miles and Wallman, 1993; Lappe, 2000). Neurones responding to optic flow also exist in the tiny brains of flies. Particularly well analysed are the approximately 60 so-called lobula plate tangential cells (LPTCs) (Hausen and Egelhaaf, 1989; Egelhaaf and Borst, 1993; Krapp, 2000; Haag and Borst, 2002a; Egelhaaf et al., 2005). They are thought to provide important components of the sensory information that enables flies to accomplish their impressive flight manoeuvres.

Virtually all investigations on LPTCs have been done on immobilised animals, because electrophysiological studies require stable recording conditions. However, flies fly or walk around in their environment when exploiting optic flow information. Their nervous system may therefore be in a different state from that when they are restrained from moving during electrophysiological experiments. Indeed, changes in the processing of visual information are indicated for the neuronal pathway mediating head movements of flies, where the motor activity state has been shown to affect optomotor head responses (Rosner et al., 2009). In locusts, changes in the responses of visual neurones associated with different behavioural states (Rowell, 1971; Rind et al., 2008) are thought to be elicited by octopamine, a biogenic amine (Bacon et al., 1995; Stern, 1999). Octopamine was also concluded to modulate the response properties of motion-sensitive neurones in bees (Kloppenburg and Erber, 1995) and LPTCs in flies (Longden and Krapp, 2009)†.

We investigated whether a state of enhanced motor activity affects LPTC responses in flies and whether such response changes can account for the observed changes in the gain of optomotor head movements (Rosner et al., 2009). While recording extracellularly or intracellularly from several LPTCs we distinguished between two spontaneously occurring states of behavioural activity by monitoring movements of the halteres: halteres are the evolutionarily transformed hindwings of flies that are known to oscillate when flies walk or fly (Sandeman and Markl, 1980). Haltere movements were used here as an indicator of a state of enhanced motor activity.

In the present account we show that the activity of LPTCs increases during periods of spontaneously occurring haltere oscillations. Whilst no response changes were reported from a previous single experiment comparing the responses to visual stimulation of one particular LPTC, the H1 cell, while the tethered fly was either at rest or flying (Heide, 1983), we found the activity of several types of LPTCs to depend on the animals' activity state. However, the increase in neural activity is too small to account for the tremendous increase in head optomotor gain during enhanced motor activity. Moreover, we conclude that direct haltere feedback is not responsible for the changes in LPTC responses. Instead we propose a central signal changing the activity of LPTCs, halteres and head optomotor gain in parallel when the fly undergoes enhanced motor activity.

MATERIALS AND METHODS

Electrophysiology

One to 10 day old female blowflies (*Calliphora vicina*, Robineau-Desvoidy 1830) were taken from the laboratory stock. The animals were mounted on custom-made holders. Wings and legs were removed and wounds sealed by bees wax. In some experiments one haltere was fixated, allowing only the other haltere to oscillate. In the other experiments both halteres were allowed to beat. A small hole was cut into the head capsule to allow access to the lobula plate. We recorded intracellularly or extracellularly from several

LPTCs using borosilicate micropipettes drawn on a microelectrode puller (Sutter Instruments P-97; Novato, CA, USA). Electrodes were filled with 1 mol l^{-1} KCl and had a typical tip resistance of 25–60 MΩ in intracellular and 1–5 MΩ in extracellular recordings. Extracellular recordings were done on LPTCs with their receptive field contralateral to the recording site. Data were recorded using a 16 bit A/D converter (DAQBoard 2000, IOtech, Cleveland, OH, USA) and analysed off-line with Matlab (MathWorks Inc., Natick, MA, USA).

Image data acquisition

To monitor haltere oscillation, flies were filmed at 250 Hz using a CMOS camera (LOGLUX i5 CL, Kamera Werk Dresden, Dresden, Germany). The images were acquired using a National Instruments frame grabber and a standard PC. The fly was illuminated by near-infrared light emitting diodes with a peak wavelength of 870 nm, which is beyond the spectral sensitivity of *Calliphora* photoreceptors (McCann and Arnett, 1972; Hardie, 1979). The spectral sensitivity of the camera ranged up to 1000 nm. We painted the haltere tips with infrared reflecting dye to facilitate analysis of haltere movements. The two halteres can beat independently (Rosner et al., 2009) and often one haltere starts oscillating several milliseconds before the other (R.R., unpublished results). Since they do not always beat at the same time it is favourable to record oscillations of both halteres to gain information about the fly's state of motor activity. However, in several experiments space was restricted by our monitor setup, allowing us to film only one haltere. In these experiments we fixated the other haltere. Haltere beat was evaluated offline as described in detail previously (Rosner et al., 2009) and additionally by visual inspection.

Visual stimulation

Flies were mounted in front of a CRT monitor with a resolution of 640×480 pixels and a refresh rate of 240 Hz. The stimuli were programmed and presented utilising a Visage stimulus generator (Cambridge Research Systems, Cambridge, UK), Matlab and a standard PC. In some experiments the monitor was positioned symmetrically in front of the fly, enabling the stimulus pattern to span 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.). In this configuration both compound eyes were stimulated. In the remaining experiments the monitor was positioned to stimulate only one compound eye, spanning an elevation from –10 deg. (ventral) to +20 deg. (dorsal) and an azimuth from +25 deg. to +70 deg. We presented a stimulus pattern consisting of 40 randomly positioned bright dots moving in front of a dark background. When stimulating both compound eyes (or only one compound eye) dots had 2 deg. (1 deg.) horizontal and 2 deg. (1 deg.) vertical extent. The exact stimulus configuration, however, is not of special interest in the present study, because we did not want to evaluate the dependence of the responses of LPTCs on stimulus parameters, but rather analysed changes in LPTC responses dependent on motor activity.

To investigate the influence of motor activity on LPTCs at different visually evoked response amplitudes, we varied the strength of the visual stimulus by (1) presenting a dark screen (0.0 cd m^{-2}) to the fly, leaving the visual system without input, (2) presenting a motionless dot pattern or (3) presenting a moving dot pattern. Additionally, we varied the brightness of the dots to yield either very strong responses with bright dots (65 cd m^{-2}) or weak responses when displaying faint dots (ranging from 0.2 to 2.5 cd m^{-2}). The same brightness value was applied every other trial. Visual stimulation lasted 1.5 s in every instance. In between the visual stimulation sequences a dark screen was shown for 13.5 s. Identical visual stimulation conditions were repeated thus every 30 s. In the Results section we repeatedly compare responses to identical stimulus conditions. We call trials with identical stimulus conditions in the nearest possible temporal vicinity 'neighbouring trials' although strictly speaking they are separated by one trial with different stimulus conditions.

The only source of light visible to the fly was the stimulus presented on the CRT. In some experiments a faint red light source was applied to enable the experimenter to orient himself. Red light is not seen by *Calliphora* (McCann and Arnett, 1972; Hardie, 1979). We ensured that this also applied to our red light source by moving a hand-held probe within the receptive field of the respective LPTC while only the red light was switched on. We could never elicit a response in this manner.

RESULTS

We investigated activity changes in blowfly LPTCs associated with the state of motor activity. The flies were either left without any visual stimulation or were confronted with a stationary or moving pattern. LPTCs respond in a direction-selective way to motion: they are excited by motion in their preferred direction and inhibited by motion in the opposite (anti-preferred) direction. LPTCs are also known to be somewhat excited solely when light is presented to the fly as compared with complete darkness (Hengstenberg, 1982).

Whilst recording responses of LPTCs we simultaneously filmed the oscillating activity of the halteres by high speed cinematography. We used the halteres as an indicator of the motor activity state of the flies since they oscillate when flies walk or fly (Sandeman and Markl, 1980). In addition, we found the halteres to oscillate when the flies were grooming (R.R., personal observation), indicating that haltere oscillations are associated not only with locomotion but also with other kinds of motor activity.

Our conclusions are based (i) on sample intracellular records, because spontaneous haltere oscillations were too rare an event during the relatively short intracellular recordings to allow for extensive quantitative analysis, and (ii) on a quantitative analysis of the consequences of motor activity on LPTCs using data from extracellular recordings.

Haltere oscillations are accompanied by activity changes in LPTCs

When the flies were not exposed to light and were thus left without visual input, haltere oscillations were accompanied by LPTC membrane potential changes. Moreover, membrane potential changes associated with haltere beat were also often found when the flies were stimulated visually.

Fig.1A and B show examples of membrane potential changes associated with haltere beat for a particular LPTC, the so-called HS- (horizontal system) cell. HS-cells are major output elements of the neuronal network processing visual motion information (Hausen and Egelhaaf, 1989) and connect directly to descending neurones and motor neurones responsible for head movements (Strausfeld and Bassemir, 1985; Strausfeld and Seyan, 1985; Strausfeld et al., 1987). These cells respond to visual motion with graded membrane potential changes superimposed by spikes of variable amplitude. In the example traces depicted in Fig.1A and B the cell was not stimulated visually but kept in total darkness during the recording. Hence, changes in the membrane potential did not result from visual input. In Fig.1A haltere oscillations went along with a pronounced depolarisation of the cell's membrane potential and an increase in membrane potential fluctuations. After the offset

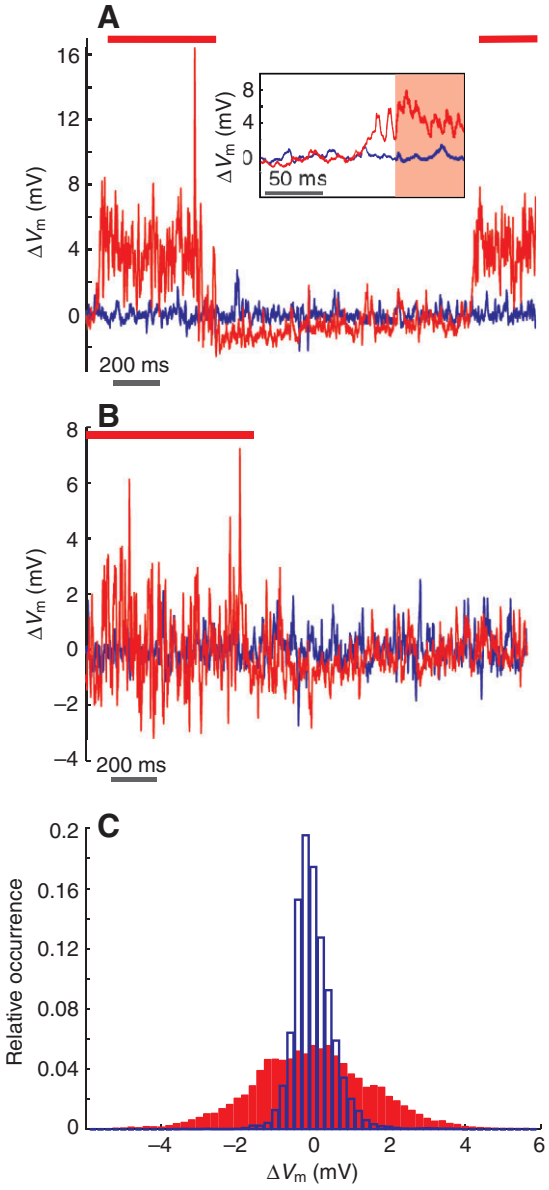

Fig. 1. Haltere activity accompanies membrane potential changes of an HS (horizontal system) cell. (A,B) The recordings were performed in complete darkness. Responses originate from the same HS cell within one recording session. Since we neither mapped the receptive fields of the recorded cell in detail nor stained them for anatomical identification, we cannot identify whether we recorded the membrane potential of an HSN (HS north) or an HSE (HS east) cell. Both halteres were filmed. In each panel two example traces are shown. For one of the traces (red) at least one haltere oscillated spontaneously during short intervals indicated by the horizontal red bars. During recording of the other trace (blue) no haltere activity occurred. The membrane potentials are given relative to the mean potential in the trial without haltere activity. Note the different scales in A and B. The inset in A zooms in on the period around the start of the second haltere beat, to illustrate the finding that the spontaneous depolarisation starts earlier than the haltere beat (indicated by the red shaded area). Also, when the haltere begins to oscillate for the first time in this trial (A), the membrane potential starts to depolarise beforehand. (C) Mean histograms of the membrane potential fluctuations averaged across sequences with (red, N=8) and without (bordered blue, N=8) haltere oscillations. The time interval with haltere oscillations defined the analysis window during which the mean membrane potential and the deviation from this mean were determined. For comparison, a neighbouring trial without haltere activity was evaluated in the same manner as a reference. For each trial a separate histogram was created specifying the occurrence of a given deviation from the mean with the area under the curve normalised to one. Subsequently, the eight histograms with/without haltere activity were averaged. The width at half height of each individual histogram obtained from a trial with haltere activity was larger than the corresponding reference width at half height of the histogram without haltere activity. This yields the difference in membrane potential fluctuations between the two activity states significant (Wilcoxon signed-rank test with α=0.05). Note that the histograms have a mean of 0 mV since here we only evaluated the deviations. We truncated histograms at –6 and +6 mV. The bin size is 0.2 mV.

of haltere activity the HS-cell hyperpolarised (Fig. 1A and B). Not in every case the mean potential changes were as pronounced as in the example traces shown in Fig. 1A. Instead the membrane potential changes comprised mainly high frequency fluctuations of the membrane potential but no obvious depolarisation (Fig. 1B). We compared the amplitude of spontaneous membrane potential fluctuations with and without haltere oscillations. Fig. 1C demonstrates that deviations from the mean membrane potential were considerably larger when halteres oscillated than when they did not oscillate.

In other types of LPTCs such membrane potential changes associated with haltere oscillations also occurred: Fig. 2A shows response traces of a VS2 or VS3 cell (vertical system cell 2 or 3) (Hengstenberg et al., 1982). Like the HS cells, VS cells are output elements of the visual system but differ from HS cells with respect to their preferred direction of motion and the location of their receptive field (Krapp, 2000). As in Fig. 1A, haltere beat is accompanied by a depolarisation and high frequency fluctuations of the membrane potential, followed by a hyperpolarisation after haltere oscillation stopped. We recorded similar changes in response during haltere oscillation in three HS cells and four VS cells.

Changes of the membrane potential were also observed in another LPTC which is not an output element of the lobula plate (Fig. 2B). This so-called CH cell (centrifugal horizontal cell) does not connect to motor neurones or descending neurones directly. Instead, the CH cell provides inhibitory input to other LPTCs (Eckert and Dvorak, 1983; Hausen, 1984; Egelhaaf et al., 1993; Warzecha et al., 1993; Gauck et al., 1997). It receives its sensitivity to motion from other LPTCs. During haltere activity the CH cell depolarised and showed an increased occurrence of IPSPs (Fig. 2B, IPSPs marked by asterisks). These membrane potential changes of the CH cell indicate that at least two of the input LPTCs are active during haltere beat. The depolarisation is probably due to input from the above-mentioned HS cell which is known to provide motion sensitivity in the ipsilateral hemisphere to CH cells (Haag and Borst, 2002b). Alternatively, the state-dependent depolarisation observed in the CH cell may be directly elicited in this cell. The IPSPs can be expected to result from inhibitory input from a spiking LPTC, the so-called element U that receives its local motion-sensitive input in the contralateral half of the brain (Hausen, 1984).

Could our results shown in Figs 1 and 2 be the consequence of artefactual membrane potential changes occurring when the fly assumes a state of high motor activity? This behavioural state could accompany small muscle contractions within the head capsule, possibly deteriorating the recording quality. Two observations argue against this explanation of our results. (1) It is highly unlikely that the membrane potential reproducibly returns to its previous level after haltere activity (see Fig. 1A). (2) The increased rate of IPSPs accompanying haltere oscillations as observed in the CH cell (Fig. 2B)

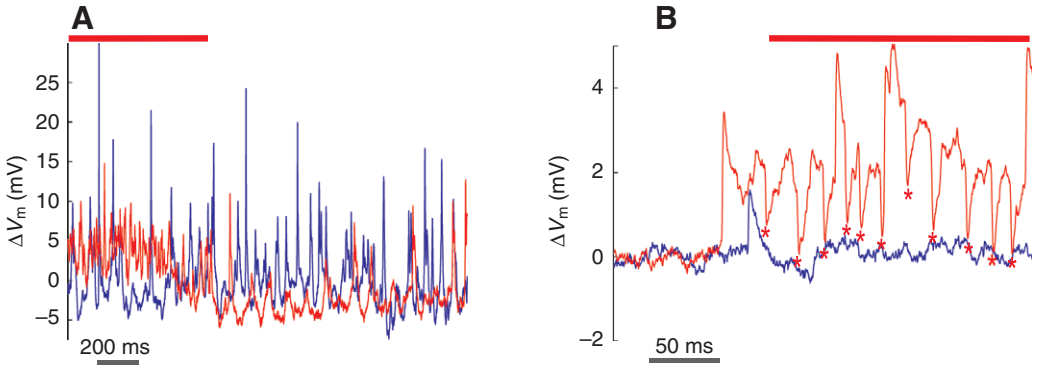

Fig. 2. Membrane potential changes associated with haltere activity in another two LPTCs (VS, vertical system and CH, centrifugal horizontal cell) recorded in total darkness. In each panel two example traces recorded in close temporal vicinity are shown. (A,B) For the red trace the ipsilateral (A) or contralateral (B) haltere oscillated spontaneously during short intervals indicated by the horizontal red bars while the other haltere was fixed. During the recording of the other trace (blue) no haltere activity occurred. The examples depicted in A and B show the responses of a VS cell and a CH cell, recorded in different animals. We did not identify whether we recorded the membrane potential of a VS2 or VS3 cell (A), or a DCH (dorsal CH) or VCH (ventral CH) cell (B). The membrane potentials are given relative to the mean potential in the trial without haltere activity. In B the continuous depolarisation and the occurrence of IPSPs (marked by asterisks) start before the onset of haltere oscillation.

cannot be the consequence of changes in the recording quality of the latter, because it is evoked by the activity of a contralateral neurone that is not penetrated with an electrode during the recording.

LPTC activity changes associated with haltere oscillation during visual stimulation

In the previous section we provided evidence for haltere beat-associated LPTC activity changes when cells were not stimulated visually. Does the elevated motor activity state associated with haltere oscillation also affect visually induced responses of LPTCs? We recorded intracellularly from one CH cell and two HS cells during visual stimulation. Sample averages of the responses of the CH cell and one of the HS cells are shown in Fig. 3. For the red traces only trials with haltere oscillations were included. During the responses shown in blue, halteres remained motionless. Both cells depolarized to a larger extent during presentation of a stationary pattern when the halteres oscillated than when they remained motionless. The HS cell also depolarised slightly more during motion in the preferred direction when the halteres oscillated than when they did not oscillate (Fig. 3A). However, during preferred-direction motion the increment in membrane depolarisation with haltere activity was not as large as that during presentation of the stationary pattern. The CH cell actually did not depolarise any more strongly during pattern motion when halteres oscillated than when they did not move (Fig. 3B). For the second HS cell we did not acquire a sufficient number of trials with haltere oscillations to calculate a reliable mean across trials. However, on a single trial basis we did not observe an obvious increment in depolarisation associated with haltere beat during visual stimulation. Nonetheless, in this HS cell too there was a change during haltere beat, i.e. the membrane potential fluctuated more strongly. During anti-preferred direction motion we observed haltere beat only in two LPTCs, a CH cell and a spiking LPTC (H1 cell). In both LPTCs haltere activity did not increase the motion-induced inhibition. Rather, the membrane potential of the CH cell as well as the spike frequency of the H1 cell were enhanced compared with the activity without haltere oscillations.

The example traces of the HS and CH cell indicate that the neuronal activity changes accompanying haltere oscillation may depend on the strength of visual stimulation: the more strongly an LPTC is stimulated by the visual stimulus the smaller the additional activation during haltere activity. To test this hypothesis we recorded extracellularly the activity of spiking LPTCs that show a similar dependence on stimulus strength to the membrane potential of HS and CH cells (Hausen, 1984). Recording extracellularly allows monitoring of the cells' responses for a longer time than could be achieved with the intracellular recordings presented so far. We analysed the influence of motor activity on the activity of four H1 cells, one V1 cell and one LPTC sensitive to back-to-front motion (possibly element U). These cells, despite having different preferred directions, have similar response properties with respect to their response mode, reaching maximum spike frequencies of more than 200 Hz when experiencing a strong motion stimulus. We applied the following visual stimuli to achieve responses of different activity levels: (1) stationary faint dots; (2) stationary bright dots; (3) moving faint dots; (4) moving bright dots. Additionally, we investigated the responses of these cells in the dark, thus leaving the visual system without input. A representative example of the responses of an H1 cell to different stimuli with and without haltere activity is shown in Fig. 4. During the recording, shown as the central trace, halteres oscillated for some time as indicated by the red marking. Obviously, in periods when the spiking activity is rather low, such as during the dark condition or the presentation of the stationary pattern, haltere activity is associated with an increase in the spike rate. The more spikes elicited by a visual stimulus the less pronounced are the consequences of haltere activity on the spike rate.

To quantify the consequences of motor activity on the responses of spiking LPTCs during the presentation of visual stimuli of different strength, we determined spike frequencies during intervals of haltere beat and compared these with spike frequencies determined for trials without haltere activity. Specifically, we determined the spike frequency contrast, i.e. the ratio of the haltere-associated increase of the response and the sum of the response amplitudes with and without haltere activity (see legend of Fig. 5). The spike frequency contrast decreases with increasing strength of the visual stimulus (Fig. 5). This holds true for all analysed spiking LPTCs, i.e. the V1 cell, the four H1 cells and the LPTC possibly being element U. Hence, the more spikes that are generated in a given stimulus situation, the smaller the relative increase of the response when the halteres beat. When the response amplitude of

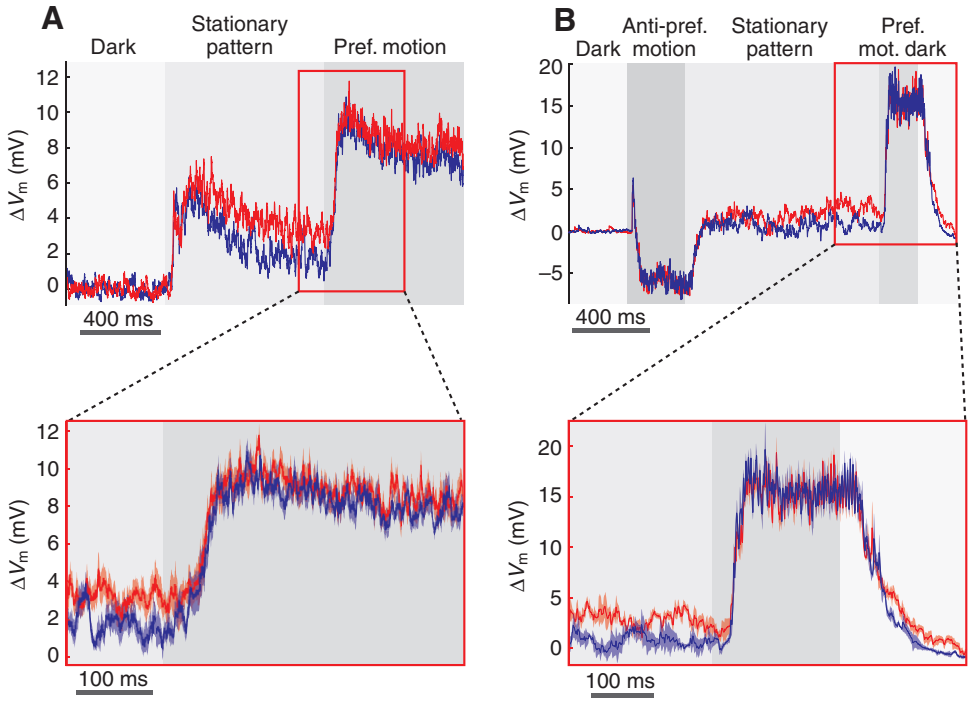

Fig. 3. Mean responses of an HS cell (A) and a CH cell (B) during visual stimulation with and without concurrent haltere beat. The reference potential (0 mV) was determined in periods without visual stimulation (dark screen) and without haltere activity. Spontaneous haltere oscillations occurred in those trials that were averaged to yield the red mean response traces. However, in none of the trials did the haltere oscillate throughout the whole recording sequence and the time duration of haltere oscillation differed in length between trials. In the lower panels those time intervals during which haltere oscillations occurred permanently in all trials contributing to the red mean trace (red rectangles in upper panels) are shown at higher magnification. These intervals are indicated in the upper panels by red rectangles. Mean responses of trials without haltere beat are shown in blue. The cells depolarised more in response to a stationary dot pattern when halteres oscillated than when they did not oscillate (compare red and blue traces). During preferred direction motion depolarisation levels of the cells were similar, irrespective of whether the haltere beat was observed. (A) Mean responses of an HS cell and standard error of the mean (shaded areas in lower panel) during haltere beat (red, *N*=13) in comparison to mean response without haltere beat (blue, *N*=13). The fly experienced a dark screen between visual stimulation sequences for 13.5 s. The last 500 ms of the foregoing dark screen period are shown in the upper panel. These 500 ms were used to determine the reference potential. As indicated, the fly was then confronted with a stationary pattern (800 ms, grey shaded) and pattern motion (700 ms, dark grey shaded) in the preferred direction of the HS cell. The filmed haltere was located ipsilateral to the recording site. The contralateral haltere was fixed. (B) Mean response traces of the CH cell and standard error of the mean (shaded areas in lower panel) when the haltere oscillated (red, *N*=5) or was still (blue, *N*=4). The fly experienced a dark screen between visual stimulation sequences (13.5 s). The last 300 ms of the foregoing dark screen period and the first 200 ms of the subsequent dark period are shown. The 300 ms at the beginning were used to determine the reference potential (0 mV). Thereafter, the fly was confronted by motion in the anti-preferred direction of the cell (300 ms), a stationary pattern (1000 ms), and motion in the preferred direction of the cell (200 ms) (different grey shading). HS cell and CH cell were recorded in different flies. CH cell same as in Fig. 2B. Note the different scales used in the four panels.

the LPTCs was already high without haltere activity, no additional increment in the spiking activity occurred when the halteres oscillated. In some trials with very weak visual stimuli (faint stationary dots) or no stimuli (dark screen) no spikes were elicited at all, even when the halteres oscillated, indicating haltere-associated membrane potential changes stayed below spike threshold. Sometimes in the dark or with weak visual stimuli spikes were only generated when the halteres oscillated.

In summary, elevated motor activity as indicated by haltere movements increases the activity of LPTCs. The response increase is more pronounced the weaker the visual stimulus. For large visually induced responses the responses do not further increase during concurrent haltere activity.

Do haltere afferences change the activity of LPTCs during haltere oscillation?

What induces the activity changes in LPTCs that accompany haltere oscillations? Haltere oscillations are detected by mechanoreceptors at the haltere base and a signal synchronous with the haltere beat is transmitted *via* afferences (Pringle, 1948; Fayyazuddin and Dickinson, 1996) providing input to motor neurones mediating head movements [physiological evidence (Huston and Krapp, 2009); anatomical evidence (Strausfeld et al., 1987)]. Motor neurones and descending neurones mediating head movements were found to be cobalt coupled to LPTCs, suggesting the existence of electrical synapses (Strausfeld and Bassemir, 1985; Strausfeld and Seyan, 1985; Strausfeld et al., 1987). Are the membrane potential changes of LPTCs during haltere oscillation caused by backflow of electrical signals from motor neurones or descending neurones into LPTCs *via* electrical synapses? If this were the case then the onset of haltere oscillations should always precede by several milliseconds the membrane potential changes observed in LPTCs.

We compared the onset of haltere oscillations with the onset of the concurrently occuring LPTC membrane potential changes for a set of intracellular recordings on a fine time scale. The inset in Fig. 1A and the recording shown in Fig. 2B illustrate that the membrane potential change can precede the onset of haltere oscillation by more than 25 ms. Although not found in every instance, this is a common phenomenon observed in membrane potential changes of LPTCs that accompany haltere oscillation. The large delay between the onset of

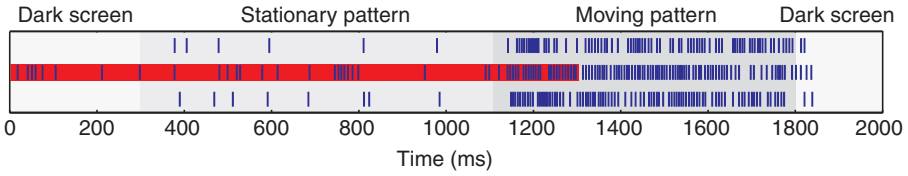

Fig. 4. Raster plot of spikes of an H1 cell indicating elevated spike rate during haltere oscillation. Each vertical line depicts the occurrence of an action potential. Traces show neighbouring trials with (central trace) and without (top and bottom trace) haltere beat for the purpose of comparison. During recording of the central trace haltere activity occurred as indicated in red. In the first 300 ms the monitor was dark. Then a stationary pattern was shown on the monitor as indicted by the grey shaded area. Subsequently (dark grey shaded area) the pattern was moving in the preferred direction of the cell. In the last 200 ms the monitor was dark again. The haltere ipsilateral to the input region of the recorded cell was filmed, the other haltere was fixed. The spike rate was elevated when the observed haltere oscillated and no visual stimulus or a stationary pattern was applied. When a motion stimulus was shown there was no obvious increase in spike frequency during haltere beat compared with the responses without haltere oscillation.

haltere oscillation and the onset of membrane potential changes renders the hypothesis that afferences signalling haltere movement are responsible for membrane potential changes in LPTCs associated with haltere movements implausible. Instead, our findings suggest a central signal that eventually leads in parallel to both membrane potential changes in LPTCs and haltere oscillations (see Discussion).

DISCUSSION

In the present account we have shown that the membrane potential and the spiking activity in several blowfly motion-sensitive LPTCs change when the halteres oscillate spontaneously. Halteres are the evolutionarily transformed hindwings of dipteran flies. They oscillate when flies walk or fly (Sandeman and Markl, 1980) indicating that the observed LPTC activity changes are related to motor activity. The membrane potential changes associated with haltere beat comprise high frequency fluctuations and depolarisations. We often found the membrane potential changes to precede the start of haltere oscillation, suggesting that the haltere oscillations are not directly responsible for the membrane potential changes in LPTCs. The relative increase in the spiking activity of LPTCs and the amount of depolarisation accompanying haltere activity was more pronounced the weaker the concurrent visual stimulation.

At first sight, our results seem to contradict an earlier investigation of the influence of motor activity of flies on the spiking activity of a particular LPTC, the H1 cell (Heide, 1983). In this review Heide reports on an experiment performed together with McCann and Foster on a tethered flying blowfly while recording the responses of an H1 cell during preferred- and anti-preferred direction motion. Their comparison of the neuronal activity during and after flight indicated no major differences in H1 cell responses (Heide, 1983). However, close inspection of the mean response averaged across four trials (Heide's figure B_2 on page 47) reveals that, in accordance with our results, the spiking activity was slightly elevated when the fly flew. Furthermore, Heide applied only very strong stimuli which, in accordance with the present results, might have reduced the detectability of changes in the neural responses during motor activity.

In the following sections we will discuss the functional significance of our results and the origin of the activity changes in LPTCs accompanying haltere oscillations.

Do LPTC activity changes account for head optomotor gain changes?

The amplitude of optomotor head responses was recently found to depend on the motor activity of the fly (Rosner et al., 2009). Fly head movements are mediated by LPTCs and they occur with two widely differing amplitudes. Large amplitude head movements occur when the fly oscillates its halteres and small amplitude head movements occur when the halteres are still. Is it possible that the elevated activity of LPTCs observed in the present study during haltere oscillation accounts for the elevated optomotor gain? This possibility can be excluded for the following reasons: when halteres beat concurrently to strong motion stimulation only a small or often

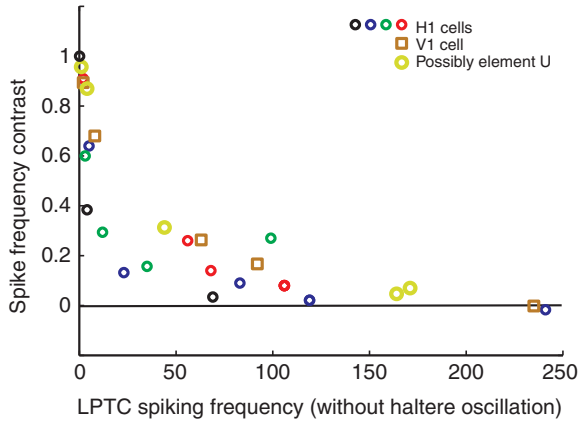

Fig. 5. The relative increase in the spike frequency associated with haltere beat is more pronounced the weaker the visual stimulus. Spike frequency contrast as a function of spike frequency without haltere beat is shown for six spiking LPTCs. We define spike frequency contrast as (spike rate with haltere beat–spike rate without haltere beat)/(spike rate with haltere beat+spike rate without haltere beat). A spike frequency contrast of 1 means that spikes were only generated during concurrent haltere oscillations. A contrast of 0 means spike frequency was identical during haltere beat in comparison to the corresponding intervals without haltere beat (indicated by black horizontal line). Spike rates were calculated for a given stimulus condition (dark monitor, moving or stationary pattern, faint or bright dots). Different spike rates were elicited by the presentation of these different conditions. Since halteres did not oscillate throughout entire trials we evaluated only those time intervals during which haltere activity occurred. Spike rate without haltere oscillations was calculated from the mean spike rate of the two trials preceding and following the 'haltere trial' in the respective time windows (defined by time interval with haltere activity). When haltere beat occurred in one of the neighbouring trials, only the trial without haltere beat was used. Symbols represent means over trials (N ranged from 1 to 18). Each cell contributes several data points because the means were calculated for each stimulus condition (for example bright stationary pattern) separately. Four data points are superimposed at the spike frequency contrast of 1. Five cells were recorded extracellularly. One intracellular H1 cell recording is included. In all but two cases the mean spike rate was higher when haltere oscillations occurred.

no increment in LPTC responses was observed, whereas the increment in optomotor head responses accompanying haltere oscillations is large even during strong visual stimulation. One might argue that even small activity changes at the level of LPTCs might evoke large changes at the level of head movements. This, however, is very unlikely because of the similarity of the stimulus–response curves of LPTCs (Hausen, 1984; Hengstenberg, 1982) and head optomotor responses (Hengstenberg, 1993). Referring to these curves, one can assume that small activity increments in LPTCs evoke small optomotor response increments. Hence, the observed changes in the LPTC responses accompanying a switch of the motor activity state are unlikely to be responsible for the gain changes of optomotor head movements. Instead, we expect these gain changes to be caused by another source downstream of the LPTCs, i.e. at the level of descending or motor neurones or at the level of muscles.

If the activity-dependent elevation of LPTC responses does not cause the high optomotor gain during motor activity, what might its functional significance be? LPTC responses are more strongly enhanced during motor activity when visual stimulation is rather weak than when it is strong. On the one hand, this reduces the output range of LPTCs in the high activity state. A smaller output range *per se* can hardly be imagined to be advantageous. On the other hand, however, the response changes associated with the elevated activity state may prepare the system to be particularly sensitive to motion stimuli. For instance, a motor or descending neurone downstream of a particular LPTC might fire with a reduced latency as a consequence of elevated overall activity in the visual system. When a particular LPTC is excited by a change in the visual surround, the spiking threshold of a downstream motor or descending neurone might be crossed earlier when the LPTC was already in a more excited state during weaker visual stimulation. As a consequence the animals may be able to react faster when they are moving actively. Energy constraints (Laughlin, 2001) may favour restriction of the elevated activity of LPTCs to periods of locomotor activity.

Mechanism mediating LPTC response changes associated with haltere beat

What causes the response elevation in LPTCs associated with haltere beat? The state dependence of LPTC responses could derive from a mechanism acting on the visual motion processing pathway prior to or directly on the LPTCs. Alternatively, the state dependence could result from back-propagation of electrical signals from the descending neurones or motor neurones *via* electrical synapses. Since at least some of the LPTCs are electrically coupled to descending neurones or neck motor neurones (Strausfeld and Bassemir, 1985; Strausfeld and Seyan, 1985; Strausfeld et al., 1987) the back-propagation hypothesis might be particularly appealing. In a previous account we suggested a central signal rather than sensory feedback to change the gain of fly head movements during motor activity (Rosner et al., 2009). Also, in locusts a central signal was found to gate sensory signals when the animals are flying (Reichert et al., 1985). In the locust, a thoracic interneurone receiving sensory input from a descending neurone additionally receives input from the central pattern generator (CPG) responsible for producing the signal for wing movements. In the fly a central signal deriving either from the brain or from a CPG located in the thorax driving haltere oscillation, wing or leg movements could cause a membrane potential change back-propagating into the LPTCs of the lobula plate.

This sort of back-propagated signal related to motor activity should only occur in LPTCs directly coupled to descending or motor neurones. However, we also found motor activity-dependent responses in LPTCs not serving as output elements of the visual system, like CH cells, H1 cells and V1 cells. In principle it is conceivable that membrane potential changes back-propagating from motor and descending neurones to output neurones of the visual system spread further across large parts of the LPTC population, since many LPTCs are electrically coupled to each other (Haag and Borst, 2005). For example, the CH cells receive their ipsilateral motion sensitivity *via* electrical synapses from the HS cells that are output LPTCs of the visual system (Haag and Borst, 2002b). However, at least two considerations challenge the 'backflow hypothesis': (1) HS and CH cells are coupled *via* dendro-dendritic connections (Haag and Borst, 2002b). An electrical signal would back-propagate from motor or descending neurones into the main output arborisation of an HS cell. These arborisations are located quite distant from the electrical synapses between HS and CH cells which reside in the dendritic tips of the HS cell in the lobula plate. Hence, the back-propagating signal can be expected to be much attenuated when reaching the electrical synapses. Therefore, the rather small motor activity-dependent response elevations in the axon of the HS cells are unlikely to elicit similar axonal depolarisations in the CH cell. (2) CH cells inhibit the H1 cell (Hausen, 1984; Haag and Borst, 2001). Since haltere activity accompanies depolarisations of the membrane potential in the CH cell, the spike rate of the H1 cell would be expected to decrease during haltere oscillations on the basis of their inhibitory input. This expectation, however, is in contrast to our results. Hence, the input from other LPTCs onto the H1 cell cannot account for increased spike rates in the H1 cell during haltere oscillations. These points argue against LPTC response elevations during haltere movements being caused by back-propagation of signals from descending and motor neurones.

An alternative explanation for the state-dependent response properties of LPTCs could be the influence of biogenic amines like octopamine on early processing stages of the visual system or directly on LPTCs. Octopamine is known to be linked to locomotor activity and grooming in flies (Yellman et al., 1997) and octopamine-like immunoreactivity is found in the optic lobes of flies (Sinakevitch and Strausfeld, 2006). In locusts an identified octopaminergic neurone dishabituates looming-sensitive visual interneurones (Bacon et al., 1995; Stern, 1999). Moreover, the response properties of photoreceptors in flies (Chyb et al., 1999), of motion-processing interneurones in bees (Kloppenburg and Erber, 1995) and more specifically of LPTCs in flies (Longden and Krapp, 2009)† have been found to change following application of octopamine. It is largely unknown on what timescale octopamine acts in sensory systems. The LPTC activity changes during haltere beat occur from one moment to another. All known octopamine receptors belong to the family of G-protein-coupled receptors (Roeder, 1999). Whether these second messenger pathways are fast enough to account for the observed LPTC activity changes remains to be elucidated.

The increase in membrane potential fluctuations observed when halteres oscillate can provide an indication of the potential site of action of a biogenic amine such as octopamine or an alternative neuromodulator: the fluctuations are reminiscent of those occurring in an LPTC when the fly is exposed to darkness and then experiences light (Hengstenberg, 1982). These light-induced activity changes are due to an increased activity of both the excitatory and the inhibitory local motion-sensitive input elements of the LPTCs. In analogy to these light-induced changes, a neuromodulator could also act to increase the activity of the input elements – directly or further upstream in the visual system. Hence, although generally speaking

we find an enhancement of LPTC responses during haltere oscillations we do not want to claim that the haltere-associated changes of the LPTC responses are due to pure excitation.

In conclusion, we have demonstrated a change in the responses of LPTCs associated with haltere oscillations. In conjunction with the available literature about fly head movements, LPTCs and the connectivity of LPTCs within the lobula plate, our results suggest the following. (1) The activity changes in LPTCs associated with haltere beat do not account for the elevated head optomotor gain described in a previous study. (2) The LPTC response elevation is not due to reafferent signals arising from haltere oscillations. Instead a central signal seems to influence LPTCs either directly or *via* elements upstream of LPTCs. This central signal is related to the motor command adjusting the motor activity state of the fly and the head optomotor gain.

†The results on response changes of LPTCs when applying octopamin (Longden and Krapp, 2009) have been published after acceptance of our manuscript.

ACKNOWLEDGEMENTS

We are grateful to anonymous referees for their helpful comments on the manuscript. This work was supported by a grant of the Volkswagen-Stiftung to A.-K.W.

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