Cross-modal influence of mechanosensory input on gaze responses to visual motion in *Drosophila*

Shwetha Mureli[1,2], Ilakkiya Thanigaivelan[1], Michael L. Schaffer[1], and Jessica L. Fox[1]*

[1]Department of Biology, Case Western Reserve University, Cleveland OH 44106-7080

[2]Present address: Department of Cardiothoracic Surgery, Stanford University, Palo Alto CA 94305-5407

*Author for correspondence: jlf88@case.edu

Key words: Halteres, gaze control, vision, multimodal integration, flight

Summary statement: Halteres, specialized fly mechanosensory organs for detecting body rotations, influence visually-guided head movements even when flies are flying straight. Removing halteres decreases head movement responses to fast-moving visual stimuli.

Abstract

Animals typically combine inertial and visual information to stabilize their gaze against confounding self-generated visual motion, and to maintain a level gaze when the body is perturbed by external forces. In vertebrates, an inner ear vestibular system provides information about body rotations and accelerations, but gaze stabilization is less understood in insects, which lack a vestibular organ. In flies, the halteres, reduced hindwings imbued with hundreds of mechanosensory cells, sense inertial forces and provide input to neck motoneurons that control gaze. These neck motoneurons also receive input from the visual system. Head movement responses to visual motion and physical rotations of the body have been measured independently, but how inertial information might influence gaze responses to visual motion has not been fully explored. We measured the head movement responses to visual motion in intact and haltere-ablated tethered flies to explore the haltere's role in modulating visually-guided head movements in the absence of rotation. We note that visually-guided head movements occur only during flight. Although halteres are not necessary for head movements, the amplitude of the response is smaller in haltereless flies at higher speeds of visual motion. This modulation occurred in the absence of rotational body movements, demonstrating that the inertial forces associated with straight tethered flight are important for gaze-control behavior. The cross-modal influence of halteres on the fly's responses to fast visual motion indicates that the haltere's role in gaze stabilization extends beyond its canonical function as a sensor of angular rotations of the thorax.

Journal of Experimental Biology • Advance article

Introduction

Animals generate optic flow on their retinas as they move. This self-generated visual motion can interfere with important visual tasks, like pursuit of a moving target. Typically, animals will stabilize their eyes against rotational visual motion that is self-generated or results from sudden perturbations, reducing blur and enhancing visual information processing. In vertebrates, the eyes rotate within the head to accomplish this stability as the head moves (Lappe et al., 1999; Miles, 1997; Paulus et al., 1984; Steinman and Collewijn, 1980). Hair cells in the inner ear sense head and body angular and linear accelerations, and provide sensory input that helps to stabilize the eye and minimize visual slip on the retina.

How do insects, without the benefit of a vestibular system, coordinate their eye movements? Insects are unable to move their eyes independently from their head, and thus the problem of gaze stabilization in insects is reduced to head stabilization. In addition to reducing motion blur, stabilizing the head also provides a common inertial reference frame (Wylie et al., 1998) for head-based sensors, including the eyes, the ocelli, and the mechanosensory antennae. In flies, body rotation velocity is measured by a pair of specialized organs known as halteres. These are reduced hindwings that oscillate in antiphase to the wings during flight (Deora et al., 2015; Pringle, 1948) and provide fast, phasic information (Fox and Daniel, 2008; Pringle, 1948) to both wing steering (Fayyazuddin and Dickinson, 1996; Fayyazuddin and Dickinson, 1999) and gaze control motoneurons (Hengstenberg, 1991; Huston and Krapp, 2009). Halteres experience small aerodynamic forces but large inertial forces, in particular a large inertial force associated with the haltere's oscillation and Coriolis forces associated with body rotations (Nalbach, 1993; Thompson et al., 2009). Although halteres function very differently from the vertebrate vestibular system, they perform some of the same functions, and are essential to maintaining flight stability in flies. Furthermore, they are external to the fly's body, making them relatively easy to experimentally manipulate or remove. At the same time, fly eyes are integrated in the head capsule and cannot move independently, and therefore eye movements can be measured simply by measuring the movement of the head.

Head movements in flies are controlled by neck motoneurons (NMNs), of which there are 22 pairs in the blowfly *Calliphora*, each connected by one or two synapses to visual sensory neurons (Milde et al., 1987; Strausfeld et al., 1987). The receptive fields of these motoneurons are similar to the receptive fields of their upstream visual sensory neurons, but often have increased sensitivity to contralateral visual motion (Huston and Krapp, 2008). In some gaze control motoneurons in larger flies (*Calliphora*), visual input can depolarize the cell membrane, but simultaneous haltere and visual input is required for the cell to reach threshold and fire action potentials (Huston and Krapp, 2009). This gating mechanism suggests that some neck muscles will not contract without simultaneous visual and haltere

Journal of Experimental Biology • Advance article

input. Notably, this gating function is apparent when stimulating the haltere with a planar, two-dimensional oscillation, as would occur when the fly is flying straight with no body rotation (Nalbach, 1993; Thompson et al., 2009). Furthermore, visual stimuli can drive activity in the steering muscles of the halteres themselves, potentially changing the haltere's movements and thus, altering the mechanosensory stimulation from the haltere sensilla (Chan et al., 1998). Therefore, the oscillations of the halteres may have an impact on gaze control even in the absence of body rotations. What are the consequences of an interaction between motion vision and haltere input for head movement behavior? Furthermore, how might the visual context change the influence of halteres on head movements?

To determine how haltere and visual inputs influence gaze control in stationary tethered flight, we observed the head movements of flying *Drosophila*. We compared intact flies to those with the entire haltere ablated to determine the effects of a loss of haltere sensory input. In recent work, we showed that removing haltere input decreases wing-steering optomotor reflex responses to wide-field motion (Mureli and Fox, 2015). At the same time, previous work showed that fixing the head has a similar dampening effect on the optomotor response (Fox and Frye, 2014). Does the decreased wing-steering optomotor response of the haltereless fly reflect an inability to properly adjust the gaze? It is possible that haltereless flies cannot move their heads, or do so in an abnormal way, and thus their wing optomotor responses could be diminished in the same way as responses in head-fixed flies. If haltere input is necessary for some neck muscles to contract, perhaps haltere removal impedes head movements, and the lack of head movements diminishes wing-steering via an unknown circuit. Removal of the halteres attenuates compensatory head roll against an imposed high-speed body rotation in a static visual surround (Hengstenberg, 1988; Schwyn et al., 2011), but the responses of stationary haltereless flies to visual motion have not been examined. We show that haltere influence on visually-guided gaze control can change with a changing visual stimulus, suggesting that mechanosensation plays an important role in gaze control even when head movements are driven by a purely visual input.

Materials and methods

Animals and technique

Female *Drosophila melanogaster* Meigen, 3–5 days post-eclosion, were reared from a colony of wild-caught flies (Card and Dickinson, 2008). Flies were cold-anesthetized and tethered to tungsten pins (Fig. 1). In some flies, we removed the entire haltere on both sides using fine forceps (Fine Science Tools, Foster City, CA). As a control manipulation, we repeated one of our experiments on another group of flies in which both halteres were glued to the thorax. Flies were allowed to recover for 30 minutes and were placed in the center of a cylindrical green LED flight arena, as described previously (Reiser and Dickinson, 2008). To examine behavior in non-flying flies, we placed a clear glass coverslip underneath the fly's tarsi to suppress flight initiation (Krämer and Markl, 1978). In all experiments, animals flew a maximum of three trials.

Head angle measurements

An infrared (IR)-sensitive Basler A601f camera with a 94 mm zoom lens (Edmund Optics, Barrington, NJ, USA) was mounted on a micromanipulator above the arena (Fig. 1). An IR LED was placed below the fly and images were captured using custom Matlab (The Mathworks, Natick, MA) software. The IR LED was turned on by an electrical relay at the onset of the visual stimulus, providing a means of video and data synchronization. The frame rate was controlled by a 5V pulse from a waveform generator (Hewlett-Packard 33120A, Palo Alto, CA, USA), and this 5V pulse, along with other data, was recorded at 1000 Hz by a data acquisition board (National

Instruments data acquisition PCI card, Austin, TX, USA) for frame synchronization. We measured the head yaw angle during each frame and aligned these data with the visual stimuli using the synchronizing pulse that triggered the camera shutter. In this way, we captured the head yaw angle and the position of the stimulus pattern at each frame. Head angle was measured from video collected at 50 frames s^{-1} using custom Matlab software (available from the authors upon request). This software made use of Matlab's Image Processing toolbox to perform binary large object (BLOB) analysis on the silhouette of the fly's head. This software automatically measured the position, size, and angle of the head in each frame. During stimulus presentation (constrained to visual yaw), the flies' heads were generally stable in the roll and pitch axes, and the software flagged any frames in which the head changed size or shape, as would occur during a roll or pitch movement.

Visual stimuli

We used two visual stimuli to test the effects of haltere removal on visually-mediated head angle responses: a wide-field panorama in which the entire visual scene moved simultaneously, and a small moving figure (a 30°-wide vertical bar) that was presented on a stationary wide-field background (these stimuli were identical to those in Fox et al., 2014). Both the figure and the wide-field panorama were composed of a random pattern of vertical stripes, such that the motion of either stimulus component provided sufficient visual contrast to stimulate the motion vision pathway. The stripes were placed such that no more than four adjacent columns of LEDs were on or off, and therefore removing any salient figures from the wide-field pattern. The "on" pixels were turned to the maximum luminance (72 cd m^{-2}), creating high contrast between on and off LEDs (maximum relative contrast 93%) (Reiser and Dickinson, 2010).

In each experiment, the pattern was moved laterally around the LED arena, simulating yaw movement. We moved the pattern in both directions in front of the fly using a triangle-wave motion. This motion oscillated the visual pattern for 0.5s in each direction, creating a 1 Hz signal. The amplitude of this signal was varied between 30° and 240° in some experiments, resulting in an overall faster or slower movement across the arena.

Analysis

Stimuli (either figure or wide-field pattern) were moved by triangle-wave functions, producing robust, approximately sinusoidal responses in intact control flies (Fig. 4A). To measure the amplitude of the responses, we fit a sine wave to each fly's head angle measurement using an error-minimization function (Fig. 4B). We report the amplitude of this fitted sine wave as the amplitude of each fly's response. A negative amplitude indicates a shift in phase between the fly's response and the fitted sine wave. Because the amplitudes were not normally distributed, we used the Wilcoxon rank-sum test to compare responses between groups.

We noted that in some cases, the fly's head angle appeared to be unrelated to the visual stimulus (Fig. 4B). We measured the correlation between the triangle-wave stimulus and the fly's response and identified any trials with a stimulus-response correlation of $|r| < 0.25$ as a low-correlation or "non-responsive" trial. This criterion was used for all experiments. In experiments with flying flies, we then analyzed the remaining higher-correlation trials using the Wilcoxon rank-sum test as above. Analyses of both the entire data set and of the high-correlation trials are shown (Figs. 4 and 5).

Results

Visually-guided head movements occur only during flight

Based on the response properties of some *Calliphora* NMNs to haltere and visual stimuli (Huston and Krapp, 2009), we predicted that flies that were not oscillating their halteres may not move their heads. If haltere oscillations are necessary for visually-guided head movements to occur, then *Drosophila* should only show such head movements during flight, because this is the only behavior in which the halteres are oscillated in this species (Geurten et al., 2014; Hall et al., 2015). By contrast, *Calliphora* show visually-guided head movements during walking behavior, and also oscillate the halteres during walking (Geurten et al., 2014; Hall et al., 2015; Sandeman and Markl, 1980). To determine if *Drosophila* show visually-guided head movements when not flying, we placed flies in a visual LED arena and stimulated the visual system with wide-field triangle-wave motion, which produces a robust head movement response in flying flies (Fox and Frye, 2014). We placed a glass coverslip under each fly to prevent it from flying and recorded head movements with an overhead video camera and custom software that measured the angle of the head with respect to the body. We observed that non-flying *Drosophila* did move their heads occasionally, but none of these flies attempted to follow the triangle-wave wide-field motion with their gaze (Fig. 2A; Movie 1). Correlation of the head movement and the wide-field stimulus was low (<0.25) for all trials (n = 18 trials in 6 flies). Notably, the flies did not hold their heads tight against the thorax, as has been observed in larger flies (Gilbert and Bauer, 1998), but rather the heads move sporadically.

Does the movement of the wings influence this change in behavior between flying and non-flying flies? Wings and halteres are homologous structures. The wings thus contain an array of campaniform sensilla (Dickinson and Palka, 1987; Gnatzy et al., 1987) that provide phasic input to wing-steering motoneurons as the wings flap (Dickinson, 1990a; Dickinson, 1990b; Fayyazuddin and Dickinson, 1999). These sensilla respond to strains on the wing resulting from aerodynamic or inertial-elastic forces (Dickerson et al., 2014; Eberle et al., 2015; Elson, 1987). Might flies rely on mechanosensory input from sensilla on the wings, as well as the halteres, to guide head movements, and might the lack of input from these sensors explain the absence of head movements in non-flying flies?

We completely ablated the wings and observed fly head movement responses to the wide-field motion stimulus while the flies were suspended in the flight arena. Although many of the wing-ablated flies did not attempt to fly, some of them did (n = 8 flies, flying in 7 of 24 trials). Flight status was determined by observing the legs, which are retracted in flight, and the halteres, which oscillate. Non-flying flies did not move their halteres nor retract their forelegs during the experiment. We again observed that non-flying flies do not attempt to follow the stimulus with their gaze (Fig. 2B; Movie 1); only one of 17 trials fell above the $|r|$

> 0.25 correlation criterion we established to determine responsiveness (Fig. 2D). However, wingless flying flies showed strong head movement responses that were all correlated with the visual stimulus, indicating that they do not require input from wing sensilla for visually-induced gaze shifts (Fig. 2C; Movie 1). These results demonstrate that *Drosophila* only show visually-guided head movements while flying (in contrast to *Calliphora*), and that mechanosensory input from the wings is not necessary for this behavior.

In these wingless flying flies, are the halteres compensating for the missing phasic information from the wing campaniform sensilla? We removed both the wings and the halteres and attempted to observe visually-guided behavior in the absence of wingbeat-synchronous mechanosensory input. Although spontaneous head movements were present in these flies, none of them retracted their legs as in flight or exhibited visually-guided head movements. Thus, we cannot determine whether the flight behavioral state or the phasic mechanosensory input is necessary for visually-guided head movements, because none of the flies attempted to fly when their wings and halteres were removed.

Spontaneous head movements occur in the absence of visual stimuli in both intact and haltereless flies

Wingless *Drosophila* will only follow visual motion with their heads when attempting to fly, suggesting that haltere oscillations might be essential to visually-guided head movements. Are halteres important for all head movements, only visually-guided head movements, or not influential on head movements at all? To answer this question, we next performed several experiments with intact flies and haltere-ablated flies.

We first determined whether haltere-ablated flying flies are able to move their heads to the same extent as intact flies in the absence of visual motion. If haltereless flies lack the physical capability to move their heads to large yaw angles, this would preclude subsequent experiments on the integration of visual and haltere information. Given that haltere input is necessary for spiking activity in a subset of neck motoneurons in *Calliphora* (Huston and Krapp, 2009), it is possible that haltere-ablated *Drosophila* would have a reduced range of yaw motion, or perhaps would not be able to move their heads at all.

We tethered intact and haltere-ablated flies to pins and placed them in the center of the arena with all of the LEDs turned on, or all off. In this way, there was no visual motion present and we could observe spontaneous head movements while the flies were flying. We observed no differences between the on or off condition and thus pooled the data from both stimuli. Although the large sample size (37,926 head angle samples over 86 trials for haltere-ablated flies, and 39,160 head angle samples over 89 trials for intact flies) resulted in statistically different distributions (Kruskal-Wallis test for equal means and two-sample F test for equal variances, $p < 0.05$), the differences in means, variances, and ranges of the head

angles were very small (Fig. 3). Most importantly, haltereless flies were observed moving their heads to large angles, showing that they have the same range of head motion as intact flies. This experiment established that haltereless *Drosophila* are still physically able to move their heads to large angles, and allowed us to ask further questions about how halteres might influence visually-guided head movements.

Haltere removal reduces head movement response to wide-field motion

Does haltere removal change the head movement response to wide-field motion? In previous work, we moved a wide-field pattern in front of intact and haltereless *Drosophila* at 30° sec^{-1} and noted that haltereless flies had a significantly smaller magnitude of modulation in their wing-steering response (Mureli and Fox, 2015). By contrast, this low stimulus speed produced similar amplitudes of response in the head angles of haltereless and intact flies (Fig. 4D; Movie 2), and a lower speed (15° sec^{-1}) resulted in nearly identical response amplitudes. Thus, though the wing-steering behavior elicited by this low-speed visual stimulus is modulated by haltere input, the head movement behavior is not. Using only low stimulus speeds, it would appear that halteres have no influence over visually-guided head movements.

Could haltere input be more influential when visual stimulus speeds are higher? As a mechanosensory organ, the haltere is able to signal to wing and neck motoneurons much more rapidly than the visual system. Previous work indicates that the visual system mediates responses to slower body rotation speeds, either perceived (when the visual stimulus is moved at a particular speed) or actual (when the body is physically rotated). In contrast, the haltere mediates responses to fast physical body rotations, and can do so in the absence of visual stimuli (Hengstenberg, 1991; Schwyn et al., 2011; Sherman and Dickinson, 2003). How do haltereless and intact flies respond when the visual stimulus speed is increased? We increased the total distance of the wide-field motion using a 1 Hz triangle wave, such that the speed of the pattern increased with increasing motion distance. Here, significant differences between intact and haltereless flies became apparent. Above 30° sec^{-1}, haltereless flies showed significantly smaller responses to faster motions than intact flies (Fig. 4D). We noted that the haltereless flies were able to move their heads up to ~8° of amplitude when moving their heads spontaneously (Fig. 3), but they did not reach that amplitude when stimulated with fast wide-field motion, in contrast to their intact counterparts (Fig. 4A, D; Movie 2). Therefore, the haltereless flies are not limited by an upper bound on their head movements, but rather are simply showing a smaller amplitude response to fast visual motion. Flies with their halteres glued to the thorax showed responses that were not significantly different from responses of haltereless flies (Fig. 4D).

We noted that intact flies modulated their responses with the speed of the visual motion, increasing the amplitude of their responses up to 180° sec^{-1} and decreasing them at

higher speeds, whereas the haltereless flies showed small responses at all speeds. A Kruskal-Wallis test indicated that intact flies' response amplitudes differ across speeds ($p < .001$), and haltereless and glued flies' responses do not ($p = 0.07$ and $p = 0.17$, respectively; Fig. 4C). Thus, in addition to decreasing the size of the head response at higher speeds of visual motion, haltere removal or silencing by gluing also decreases the variation in head response to different visual speeds, effectively making the flies insensitive to visual speed.

Are the responses of haltereless flies generally smaller, or do fewer haltereless flies respond to the visual stimulus? Trials without any head rotations, or with rotations unrelated to the stimulus, would decrease the size of the average response, even if flies responded with high amplitudes in other trials. We observed such trials in intact, haltereless, and haltere-glued flies when examining the raw traces of the head angles (Fig. 4B). Do more haltereless flies simply fail to respond to the visual stimulus, and does this explain the lower average response of this group? We measured the correlation between the visual stimulus and the head movement response in each trial. We set $|r| < 0.25$ as a criterion for labeling a given trial as low-correlation and re-analyzed the remaining high-correlation trials, removing the low-correlation responses from the data set. The percentage of trials classified as low-correlation was not different in intact and haltereless flies (Fisher's exact test, $p > 0.05$ at all speeds; Fig. 5). In contrast to the scenario proposed above-- that the low median response of haltereless flies can be explained by a high number of non-responsive trials-- we found that the differences in amplitudes of the high-correlation trials between intact and haltereless flies are even larger than the differences when considering all trials. This analysis indicates that the smaller median response amplitude of haltereless flies is due to smaller (but still correlated) responses, not due to larger numbers of flies producing a low-correlation response. Examination of raw traces of the response (Fig. 4A) shows that high-correlation trials from haltereless flies are smaller in amplitude than high-correlation trials from intact flies.

We repeated the Kruskal-Wallis test on the subset of high-correlation trials. The results of this analysis were not different from the analysis of all trials: intact flies show different amplitudes of head response at different speeds (p =.001) and haltereless and haltere-glued flies do not ($p = 0.08$ and 0.07, respectively; Fig. 4C, dashed lines).

Haltere removal does not decrease head response to moving figures

We presented intact and haltereless flies with a similar triangle-wave motion as above, but moved a small figure (a 30º-width bar composed of random stripes) against a static wide-field background (random stripes on the rest of the LEDs in the arena). Notably, although the figure is the only part of the stimulus that moves, wide-field motion on the retina will occur in equal magnitude and opposite direction to any of the fly's head movements, due to the static stripes around the arena. We found that haltere ablation does not significantly

change the amplitude of the head angle response (Fig. 6A, B), in large part because responses to figure movement were noisy for all flies. We also noted that low-correlation trials contained large, spontaneous head movements in both groups of flies, which was not observed during low-correlation trials in wide-field experiments (Fig. 4A).

When the amplitude (and thus the speed) of the figure's motion was increased, response amplitudes of both intact and haltereless flies did not change (Fig. 6B). Responses of haltereless flies were not different from responses of intact flies at any speed (Wilcoxon rank sum test, $p > 0.05$). We tested the moving figures over the same range of motion amplitudes used in the wide-field experiments (Fig. 4), but neither intact nor haltereless flies responded to figure motion faster than 120º s^{-1} (Fig. 6B). Correlations between the visual stimulus and the head response were low: most trials did not meet the $|r| > 0.25$ criterion established above, and those that did meet it were still generally low (the example high-correlation traces shown in Fig. 6A have $|r| = 0.26$ and 0.32, for the intact trials, and 0.28 for the haltereless trial; see Fig. 5 for all correlations). The fraction of trials classified as high-correlation did not differ between intact and haltereless flies at any speed (Fisher's exact test, $p > 0.05$; Fig. 5).

Because the number of high-correlation trials was so low, we compared these trials to those of the control experiment above in which no visual stimulus was shown. We measured the correlation between the head responses to a blank arena, in a separate experiment (Fig. 2), and the figure motion we provided in this experiment (Fig. 6). This fictive correlation indicates how many high-correlation trials we may expect due to chance. In the blank arena, we found 2 of 89 trials of intact flies and 0 of 86 trials of haltereless flies in which the correlation to the fictive stimulus was >0.25. We found a significantly larger fraction of high-correlation trials in some, but not all, of our figure-tracking experiments (for intact flies: 90 º s^{-1} and 120 º s^{-1}, and the dark bar at 60 º s^{-1}; for haltereless flies: 15 º s^{-1}, 120 º s^{-1}, and the dark bar at 60 º s^{-1}; Fig. 5).

In an attempt to elicit larger responses to moving figures, we showed the flies a dark bar on a bright background, predicting that the high contrast and lack of confounding self-generated wide-field motion on the retina would result in larger responses of the fly's head to the moving bar. This experiment did not result in a greater fraction of high-correlation responses (Fisher's exact test as compared to trials with a moving figure on a randomly striped background at 60º s^{-1}, $p > 0.05$ for intact and haltereless flies; Fig. 5). The responses to the dark bar were also not significantly larger than responses to the figure moving on a randomly striped background (Wilcoxon rank sum test, $p > 0.05$ when comparing intact and haltereless flies between experiments). Haltere removal did not decrease the response to the dark bar (Wilcoxon rank sum test, $p > 0.05$; Fig. 6).

Discussion

Visually-guided head movements only occur during flight, but do not require halteres

Given that haltere input is required for some *Calliphora* neck motoneurons to spike (Huston and Krapp, 2009), we predicted that removing haltere input would dampen or eliminate *Drosophila's* head movements during flight. This prediction was not supported: we did not observe any change in head angle range in haltereless flies (Fig. 3), and haltereless flies followed a slow-moving stimulus with the same robust response as intact flies (Fig. 4).

We noted that *Drosophila* that were not flying did not follow the same visual stimulus with their gaze (Fig. 2A, B). Input from wing campaniform sensilla is not necessary for visually-guided gaze control, as we observed strong head movement responses in fictively flying flies with ablated wings (Fig. 2C). The general state of flight appears to be necessary for flies to "lock" their gaze to the visual stimulus, but our experiments did not point to the halteres or the wings as essential to this behavior. In *Calliphora*, movements of the head are gated by an unidentified central input that is active during flight, and the head is locked and immobile when this central input is not active. This helps the fly to separate self-generated and external visual stimuli: when the fly is at rest, any movement on the retina must be caused by movement in the external world (Haag et al., 2010). Though a similar flight-activated central input may be at work in *Drosophila*, their heads do make spontaneous saccades when the flies are not flying (Fig. 2).

Haltere removal influences head movements even when the fly is not rotating

Along with previous work (Fayyazuddin and Dickinson, 1999; Huston and Krapp, 2009; Mureli and Fox, 2015), our results further demonstrate the power of haltere input to modulate fly behavior in the absence of body rotations. In our experiments, the halteres never experienced the Coriolis forces associated with body rotations, but removal of the phasic input from the haltere's oscillation was sufficient to diminish the fly's head responses when the visual stimulus was moving at high speeds (Fig. 4). The role of the haltere in modulating responses to visual stimuli thus goes beyond its function as a body rotation velocity sensor.

Do the planar haltere oscillations occurring in tethered flight serve as a signal to the fly's brain that it is flying? Though haltere input is distinct from the flight-related central input to the neck motoneurons of larger flies (Haag et al., 2010), the movement of the halteres could cause some of the state-dependent modulations observed in the *Drosophila* visual system during flight (Maimon et al., 2010; Suver et al., 2012). If this was the case, the behavior of haltereless and non-flying *Drosophila* would be similar. However, haltereless flies are still able to follow visual stimuli with their heads, albeit in an attenuated way, whereas non-flying flies make no attempt to fixate the visual stimulus with their gaze. Furthermore, state-dependent changes in the visual system occur when fruit flies are walking

and not oscillating their halteres (Chiappe et al., 2010), indicating that halteres are not necessary for the behaviorally-gated changes in visual processing. Therefore, the oscillation of the halteres does not appear to be a relevant signal to the *Drosophila* brain that primes it for responses to fast-moving stimuli during flight.

Halteres influence wide-field stabilization behaviors to a greater extent than figure-tracking behaviors

In combination with previous work (Mureli and Fox, 2015), these results demonstrate further that haltere removal has little to no influence over *Drosophila's* ability to follow a small moving figure by means of wing-steering maneuvers or gaze shifts. Head rotations in response to moving figures are generally not very large (Fox and Frye, 2014; Fig. 6). One possible reason for this is that the spatial resolution of *Drosophila* eyes does not vary across the eye surface, and thus there is no increase in information processing when the fly rotates its head toward an object (Geurten et al., 2014; Land and Eckert, 1985). This is in contrast to other insects that possess an acute zone of higher spatial resolution and turn their heads towards objects of interest to increase their visual resolution of the object (Gonzalez-Bellido et al., 2011; Olberg et al., 2007). Head movements in *Drosophila* are thus used primarily to stabilize wide-field motion, rather than tracking moving figures (Fox and Frye, 2014). These results also suggest that halteres contribute more to reflexive, "inner-loop" behaviors than to voluntary "outer-loop" behaviors (Krapp and Wicklein, 2008; Mureli and Fox, 2015). These differences in head movement behaviors between *Drosophila* and other flies, in combination with differences in haltere movement behaviors (Hall et al., 2015), also open the possibility that haltere inputs to neck motoneurons might differ significantly between fly taxa.

What is the sensory effect of *Drosophila* head movements on the visual input? Our data suggest that the effect might be small. The head movements observed here reached ~10° at maximum, even when the amplitude of the pattern's motion was triple that or greater. These head movements then cannot completely stabilize the fly's visual field against pattern motion in this tethered flight setup. However, because head movements are typically accompanied by wing-steering movements in purely wide-field surrounds (Duistermars et al., 2012; Liske, 1977; Schilstra and van Hateren, 1998), it is possible that head movements are accessories to larger stabilizing body movements. Head movements could be used to compensate in part for the fly's posterior blind spot, but this blind spot is approximately 40° in width (20° for each eye's visual field; Heisenberg and Wolf, 1984), which would be too large for the small head movements observed here to adequately cover.

Haltere input helps flies respond to fast motion, independent of modality

When the visual wide-field stimulus is moved relatively slowly, there is no difference in the head movement responses of intact and haltereless flies. At higher visual motion speeds, however, the responses of haltereless flies decrease in amplitude (Fig. 4), up to the speeds at which both intact and haltereless flies show low-amplitude responses. This experiment was inspired by, and the results are in keeping with, previous findings that halteres generally mediate responses to faster body motions and the visual system mediates responses to slower visual motion. Haltere information is combined with slower feedback information from the visual system to provide a robust mechanism for adjusting the head over a broad range of body rotation velocities (Hengstenberg, 1988; Schwyn et al., 2011). This gaze-adjustment mechanism is similar in its range fractionation capacity to the mechanism that combines haltere and visual information for wing-steering behavior (Sherman and Dickinson, 2003).

What is novel about the results here is that this range fractionation persists in a cross-modal way. Halteres influence gaze control responses at high visual motion speeds, even when the fly's body is stationary (Fig. 4). The results shown here are similar to recent findings suggesting that proprioceptive haltere input can dynamically adjust visually-stimulated motor commands to the wings (Bartussek and Lehmann, 2016; Mureli and Fox, 2015). As is the case in wing-steering behaviors, it is possible that the change in head movements following haltere removal is a result of a change in phase-dependent efficacy of the muscles (Bartussek and Lehmann, 2016; Lehmann and Bartussek, 2016); however, it is not known how the spike phase of the haltere input influences neck muscle function.

Neural integration of haltere and visual information in the NMNs is nonlinear (Huston and Krapp, 2009). However, the overall behavior of blowflies experiencing body roll in a stationary visual surround is generally linear (Schwyn et al., 2011). Our data demonstrate that this linearity may persist in this cross-modal stimulus paradigm. Head movements in *Drosophila* are not eliminated or even slowed by haltere removal; rather, they are simply made smaller, consistent with a linear combination of haltere and visual inputs to produce head movement behavior.

How are visual and haltere inputs integrated in other neurons in the fly's central nervous system? In blowflies, lobula plate tangential cells (LPTCs) in the visual system show an increase in their membrane potential when the halteres are actively beating, but the small increase does not likely account for the radical change in head optomotor gain between blowflies that are moving their halteres and blowflies that are not (Rosner et al., 2010). Thus, the results we see here are not likely to be caused by a gain change in visual neurons during haltere oscillation. An identified neck motoneuron (VCNM) in blowflies integrates numerous signals from halteres, antennal mechanoreceptors, and visual neurons, as well as a central neuron signaling flight activity (Haag et al., 2010). This central neuron provides stronger

input to the VCNM than the input from the campaniform sensilla of the haltere (Haag et al., 2010), and this is likely to explain the lack of head movement in non-flying *Drosophila* and robust head movements in flying wingless or haltereless *Drosophila* (Fig. 2). Our experiments demonstrate that removal of the haltere in flying flies diminishes, but does not eliminate, the head movement response driven by VCNM (if present in *Drosophila*) or other neck motoneurons. Thus, our behavioral results are consistent with the electrophysiological results in larger flies (Haag et al., 2010).

Efference copy modulation of haltere input

Flies, like all moving animals, face the challenging problem of distinguishing self-generated body movements from externally-imposed perturbations. In the *Drosophila* visual system (horizontal cells of the lobula plate), modulation of the membrane voltage occurs during self-generated attempted yaw saccades. This modulation is a flexible efference copy, and is modified in magnitude and direction to match the voltage change that would be expected as a result of self-generated wide-field motion (the reafference), and occurs during saccades, even in flies that are blinded (Kim et al., 2015). The exact source of this efference copy is still unknown, but its effects would be sufficient to cancel the expected visual input resulting from saccade-associated head turns. Recent evidence shows that this efference copy input is quantitatively modulated to silence visual input in specific directions of rotation while maintaining sensitivity in other directions (Kim et al., 2017).

The halteres are necessary for full-amplitude gaze stabilizing responses to high-speed visual motion. We might predict that haltereless flies would experience a mismatch between the expected head movement, as predicted by the efference copy, and the actual visual feedback, which would be smaller than expected due to the smaller head movements seen in haltereless flies. This would be difficult to test behaviorally, however, because interpretations of tethered flight behaviors are necessarily limited, and because haltereless flies cannot fly freely.

Alternatively, the efference copy may modulate input from both sensory systems. If the flexible inhibition provided by the efference copy is available to modulate neurons of the visual system, it may also be available to neurons of the haltere system. Efference copy modulation of haltere sensory input could occur at any point: it could change the tension on the haltere muscle (Chan et al., 1998), raise firing thresholds in haltere primary sensory neurons, or inhibit neck motoneuron responses to haltere input. Our results show that removing haltere input results in smaller head movement responses to visual stimuli. In intact flies executing self-generated turns, compensatory head movements are not observed (Geiger and Poggio, 1977). An efference copy acting on the haltere input during a self-generated body

rotation could decrease the compensatory optomotor response we observe in tethered flies and potentially account for the difference in behavior between tethered and freely-flying flies.

Acknowledgements

We thank Mark Willis, Alexandra Yarger, and Nick Kathman for helpful discussion, and Michael Rauscher for assistance in data collection.

Competing interests

No competing interests declared.

Author contributions

SM and JLF designed experiments. IT and SM collected data. IT, SM, and JLF analyzed data. MLS wrote custom software for automatic head-angle tracking. JLF wrote the manuscript with input from all authors.

Funding

This work was funded by laboratory start-up funds from Case Western Reserve University, a Konishi Award from the International Society for Neuroethology, and Air Force Office of Scientific Research grants [FA9550-14-0398 and FA9550-16-1-0165] to JLF.

Data availability

Data for this project has been deposited in Dryad: doi:10.5061/dryad.v6q20.

References

Bartussek, J. and Lehmann, F.-O. (2016). Proprioceptive feedback determines visuomotor gain in *Drosophila*. *R Soc Open Sci* **3**, 150562.

Chan, W. P., Prete, F. and Dickinson, M. H. (1998). Visual input to the efferent control system of a fly's "gyroscope." *Science* **280**, 289–292.

Chiappe, M. E., Seelig, J. D., Reiser, M. B. and Jayaraman, V. (2010). Walking modulates speed sensitivity in *Drosophila* motion vision. *Curr Biol* **20**, 1470–1475.

Deora, T., Singh, A. K. and Sane, S. P. (2015). Biomechanical basis of wing and haltere coordination in flies. *Proc Natl Acad Sci USA*.

Dickerson, B. H., Aldworth, Z. N. and Daniel, T. L. (2014). Control of moth flight posture is mediated by wing mechanosensory feedback. *J Exp Biol* **217**, 2301–2308.

Dickinson, M. H. (1990a). Linear and nonlinear encoding properties of an identified mechanoreceptor on the fly wing measured with mechanical noise stimuli. *J Exp Biol* **151**, 219–244.

Dickinson, M. H. (1990b). Comparison of encoding properties of campaniform sensilla on the fly wing. *J Exp Biol* **151**, 245–261.

Dickinson, M. H. and Palka, J. (1987). Physiological properties, time of development, and central projection are correlated in the wing mechanoreceptors of *Drosophila*. *J Neurosci* **7**, 4201–8.

Duistermars, B. J., Care, R. A. and Frye, M. A. (2012). Binocular interactions underlying the classic optomotor responses of flying flies. *Front Behav Neurosci* **6**, 6.

Eberle, A. L., Dickerson, B. H., Reinhall, P. G. and Daniel, T. L. (2015). A new twist on gyroscopic sensing: body rotations lead to torsion in flapping, flexing insect wings. *J R Soc Interface* **12**, 20141088.

Elson, R. C. (1987). Flight motor neurone reflexes driven by strain-sensitive wing mechanoreceptors in the locust. *J Comp Physiol A* **161**, 747–760.

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.

Fayyazuddin, A. and Dickinson, M. H. (1999). Convergent mechanosensory input structures the firing phase of a steering motor neuron in the blowfly, *Calliphora*. *J Neurophysiol* **82**, 1916–1926.

Fox, J. L. and Daniel, T. L. (2008). A neural basis for gyroscopic force measurement in the halteres of *Holorusia*. *J Comp Physiol A* **194**, 887–897.

Fox, J. L. and Frye, M. A. (2014). Figure–ground discrimination behavior in *Drosophila*. II. Visual influences on head movement behavior. *J Exp Biol* **217**, 570–579.

Geiger, G. and Poggio, T. (1977). On head and body movements of flying flies. *Biol Cybern* **25**, 177–180.

Geurten, B. R. H., Jähde, P., Corthals, K. and Göpfert, M. C. (2014). Saccadic body turns in walking *Drosophila*. *Front Behav Neurosci* **8**,.

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.

Gnatzy, W., Grünert, U. and Bender, M. (1987). Campaniform sensilla of *Calliphora vicina* (Insecta, Diptera). I. Topography. *Zoomorphology* **106**, 312–319.

Gonzalez-Bellido, P. T., Wardill, T. J. and Juusola, M. (2011). Compound eyes and retinal information processing in miniature dipteran species match their specific ecological demands. *Proc Natl Acad Sci U S A* **108**, 4224–9.

Haag, J., Wertz, A. and Borst, A. (2010). Central gating of fly optomotor response. *Proc Natl Acad Sci U S A* **107**, 20104–9.

Hall, J. M., McLoughlin, D. P., Kathman, N. D., Yarger, A. M., Mureli, S. and Fox, J. L. (2015). Kinematic diversity suggests expanded roles for fly halteres. *Biol Lett* **11**, 20150845.

Heisenberg, M. and Wolf, R. (1984). *Vision in Drosophila: Genetics of Microbehavior*. Berlin: Springer-Verlag.

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.

Huston, S. J. and Krapp, H. G. (2008). Visuomotor transformation in the fly gaze stabilization system. *PLoS Biol* **6**, e173.

Huston, S. J. and Krapp, H. G. (2009). Nonlinear integration of visual and haltere inputs in fly neck motor neurons. *J Neurosci* **29**, 13097–13105.

Kim, A. J., Fenk, L. M., Lyu, C. and Maimon, G. (2017). Quantitative predictions orchestrate visual signaling in Drosophila. *Cell* **168**, 280-294.

Kim, A. J., Fitzgerald, J. K. and Maimon, G. (2015). Cellular evidence for efference copy in *Drosophila* visuomotor processing. *Nat Neurosci* **18**, 1247–1255.

Krämer, K. and Markl, H. (1978). Flight-inhibition on ground contact in the American cockroach, *Periplaneta americana*—I. Contact receptors and a model for their central connections. *J Insect Physiol* **24**, 577–586.

Krapp, H. and Wicklein, M. (2008). Central processing of visual information in insects. In *The senses: A comprehensive reference* (ed. Masland, R.) and Albright, T. D., pp. 131–204. San Diego: Academic Press.

Land, M. F. and Eckert, H. (1985). Maps of the acute zones of fly eyes. *J Comp Physiol A* **156**, 525–538.

Lappe, M., Bremmer, F. and van den Berg, A. V (1999). Perception of self-motion from visual flow. *Trends Cogn Sci* **3**, 329–336.

Lehmann, F.-O. and Bartussek, J. (2016). Neural control and precision of flight muscle activation in *Drosophila*. *J Comp Physiol A* **203**, 1–14.

Liske, E. (1977). The influence of head position on the flight behaviour of the fly, *Calliphora* erythrocephala. *J Insect Physiol* **23**, 375–379.

Maimon, G., Straw, A. D. and Dickinson, M. H. (2010). Active flight increases the gain of visual motion processing in *Drosophila*. *Nat Neurosci* **13**, 393–399.

Milde, J. J., Seyan, H. S. and Strausfeld, N. J. (1987). The neck motor system of the fly *Calliphora erythrocephala* II. Sensory organization. *J Comp Physiol A* **160**, 225–238.

Miles, F. A. (1997). Visual stabilization of the eyes in primates. *Curr Opin Neurobiol* **7**, 867–871.

Mureli, S. and Fox, J. L. (2015). Haltere mechanosensory influence on tethered flight behavior in *Drosophila*. *J Exp Biol* **218**, 2528–37.

Nalbach, G. (1993). The halteres of the blowfly *Calliphora*. *J Comp Physiol A* **173**, 293–300.

Olberg, R. M., Seaman, R. C., Coats, M. I. and Henry, A. F. (2007). Eye movements and target fixation during dragonfly prey-interception flights. *J Comp Physiol A* **193**, 685–93.

Paulus, W. M., Straube, A. and Brandt, T. H. (1984). Visual stabilization of posture: physiological stimulus characteristics and clinical aspects. *Brain* **107**, 1143–1163.

Pringle, J. W. S. (1948). The gyroscopic mechanism of the halteres of Diptera. *Philos Trans R Soc L B Biol Sci* **233**, 347–384.

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

Reiser, M. B. and Dickinson, M. H. (2010). *Drosophila* fly straight by fixating objects in the face of expanding optic flow. *J Exp Biol* **213**, 1771–1781.

Rosner, R., Egelhaaf, M. and Warzecha, A.-K. (2010). Behavioural state affects motion-sensitive neurones in the fly visual system. *J Exp Biol* **213**, 331–8.

Sandeman, D. C. and Markl, H. (1980). Head movements in flies (*Calliphora*) produced by deflexion 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.

Schwyn, D. A., Heras, F. J. H., Bolliger, G., Parsons, M. M., Krapp, H. G. and Tanaka, R. J. (2011). Interplay between feedback and feedforward control in fly gaze stabilization. *Prepr 18 IFAC World Congr* 9674–9679.

Sherman, A. and Dickinson, M. H. (2003). A comparison of visual and haltere-mediated equilibrium reflexes in the fruit fly *Drosophila melanogaster*. *J Exp Biol* **206**, 295–302.

Steinman, R. M. and Collewijn, H. (1980). Binocular retinal image motion during active head rotation. *Vision Res* **20**, 415–429.

Strausfeld, N. J., Seyan, H. S. and Milde, J. J. (1987). The neck motor system of the fly *Calliphora erythrocephala* I. Muscles and motor neurons. *J Comp Physiol A* **160**, 205–224.

Suver, M. P., Mamiya, A. and Dickinson, M. H. (2012). Octopamine neurons mediate flight-induced modulation of visual processing in *Drosophila*. *Curr Biol* **22**, 2294–2302.

Thompson, R. A., Wehling, M. F., Evers, J. H. and Dixon, W. E. (2009). Body rate decoupling using haltere mid-stroke measurements for inertial flight stabilization in Diptera. *J Comp Physiol A* **195**, 99–112.

Wylie, D. R. W., Bischof, W. F. and Frost, B. J. (1998). Common reference frame for neural coding of translational and rotational optic flow. *Nature* **392**, 278–282.

Figures

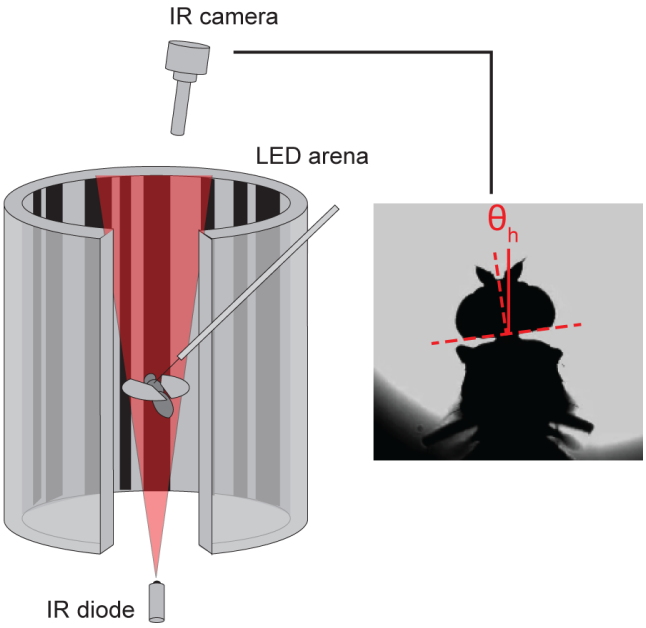

Figure 1. Experimental setup. Flies are rigidly tethered to pins and surrounded by an arena of green LED panels on which various stimuli can be displayed. A camera above the fly records an image of the fly's head (inset), and the angle of the head (θ_h) is analyzed by custom tracking software.

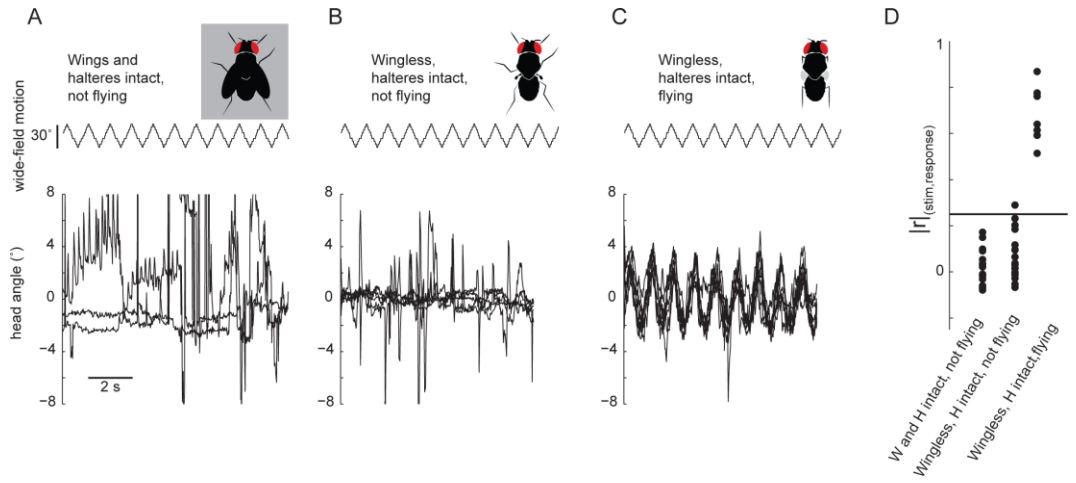

Figure 2. Flies that are not flying do not show visually-guided head movements. A) Flies standing on a glass coverslip (indicated by gray box under fly) show sporadic head movements that are not related to the visual stimulus (n = 6 flies). B) Wingless flies that are suspended, but not attempting to fly, also show sporadic head movements (n = 8 flies, 17 trials). C) Wingless flying flies, as identified by oscillating halteres and body posture, show visually-guided head movements (n = 8 flies, 7 trials). D) Correlations of the head movement response with the visual stimulus for the flies shown in Fig. 2A-C. Only flying flies showed head movements correlated with the stimulus. Black line indicates the $|r|>0.25$ criterion level for responsiveness.

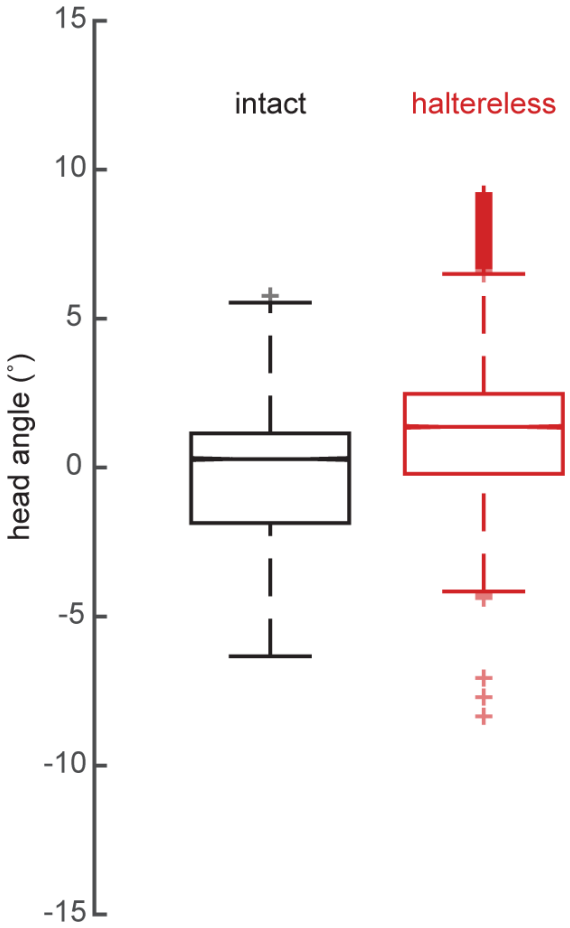

Figure 3. Distributions of spontaneous head movement angles in the absence of visual stimuli exhibited by intact (black) and haltereless flies (red) are similar. Removing the halteres does not impair the fly's ability to move its head. Haltereless flies have similar mean head angles and similar range of head movements compared to intact flies. Data shown are pooled from experiments with all visual LED panels turned on and all panels turned off. Although the large sample size (n = 15 flies in each group: 37,926 head angle samples over 86 trials for haltere-ablated flies, and 39,160 head angle samples over 89 trials for intact flies) resulted in statistically different distributions (Kruskal-Wallis test for equal means and two-sample F test for equal variances, $p < 0.05$), the differences in means, variances, and ranges of the head angles were very small.

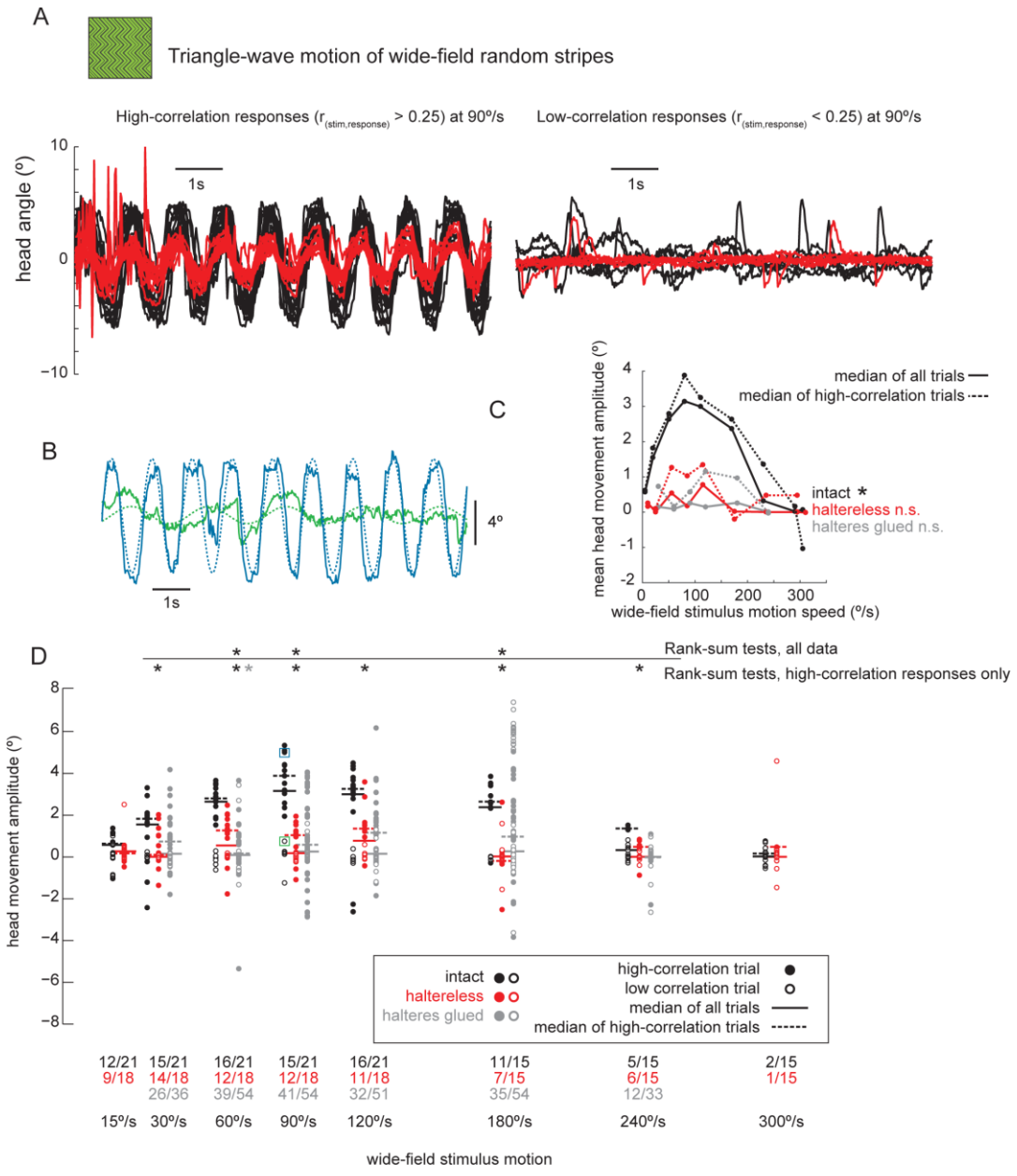

Figure 4. Haltereless flies show smaller response amplitudes to higher speeds of wide-field visual motion. A) High-correlation (left) and low-correlation (right) trials for intact (black) and haltereless (red) flies. Response amplitudes are lower for all haltereless flies than for all intact flies in high-correlation trials. B) Examples of high-correlation (blue) and low-correlation (green) trials from intact flies and their respective fitted sine waves (dashed lines). Amplitudes of these example traces are indicated by squares in D. C) Median amplitudes of intact (black), haltereless (red), and haltere-glued (gray) trials. A Kruskal-Wallis test indicates that intact flies vary their response amplitudes with varying speeds (asterisk), whereas haltereless and haltere-glued flies do not (n.s.). This result was consistent when including all

trials (solid lines) or when including only high-correlation trials (dashed lines). Medians in this figure are derived from the data shown in D. D) Response amplitudes of intact flies (black), haltereless flies (red), and flies with halteres glued to the thorax (gray) at increasing angles of wide-field motion at a constant pattern velocity. Filled circles indicate trials with a high correlation between visual stimulus angle and head movement response ($|r| > 0.25$), and open circles indicate low-correlation trials. Solid horizontal lines indicate the median head movement amplitude of all trials; dashed lines indicate median head movement amplitude of only the high-correlation trials. Intact flies had higher amplitude responses than haltereless flies at 60, 90, and 180 ° sec^{-1} when analyzing all trials, and at 30, 60, 90, 120, 180, and 240 ° sec^{-1} when analyzing only high-correlation trials (Wilcoxon rank-sum test, $p < 0.05$; black asterisks). Haltereless flies and flies with halteres glued to the thorax were not significantly different at any speed when analyzing all trials, and at only one speed when analyzing high-correlation trials (gray asterisk). Green and blue boxes indicate the data points derived from the traces in B. The numbers below each data set show the number of high-correlation trials as a fraction of the total number of trials; this fraction did not differ between treatment groups (Fisher's exact test, $p > 0.05$). Each fly flew three trials. Three data points with amplitude above 8 and below -8° (one haltere-glued trial at 60° sec^{-1} and two haltere-glued trials at 240° sec^{-1}) are included in the analysis but not shown, for visibility on these axes.

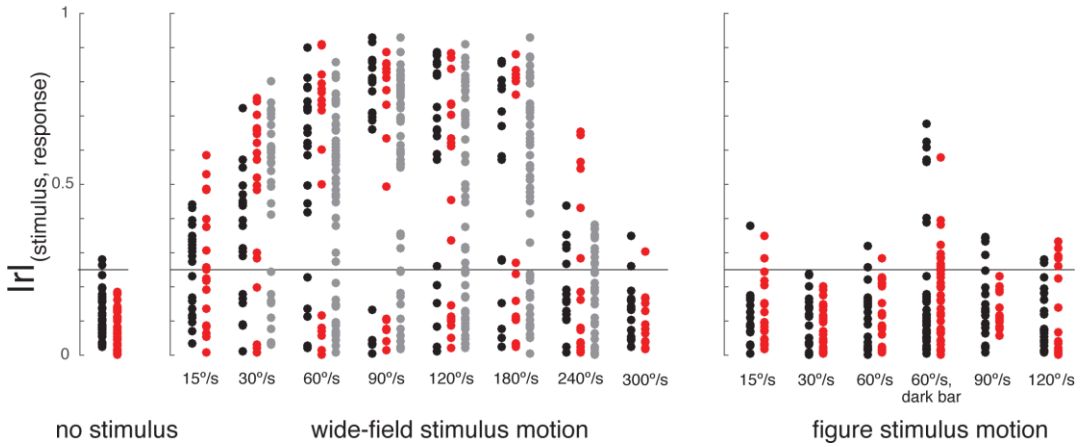

Figure 5. Correlations between stimulus and response for experiments with no visual stimulus (left), wide-field stimuli (middle), and moving figures (right) for intact (black), haltereless (red), and haltere-glued flies (gray). A) Correlations between head responses of flies in an arena with all lights on or off (see Fig. 2) and a fictive triangle-wave motion stimulus as used in the experiments in Figs. 4 and 6. This provided an estimate of the number of high-correlation trials that may be expected to occur due to chance. B) Correlations between wide-field visual stimulus and head movement responses. C) Correlations between figure stimulus and head movement responses. There were no differences in stimulus-response correlations between intact and haltereless flies at any speed, for any stimulus (Wilcoxon rank-sum test, $p > 0.05$). Responses to wide-field motion were more strongly correlated than responses to figure motion for all speeds and both groups of flies (Wilcoxon rank-sum test, $p < 0.05$). Though there were some responses of intact flies to the dark bar stimulus that were strongly correlated, these trials were overall not more strongly correlated than intact flies' responses to the randomly striped figure at the same speed. Black lines indicate the $|r|>0.25$ criterion level for responsiveness. Numbers of trials for these experiments are the same as in Figs. 2, 4, and 6.

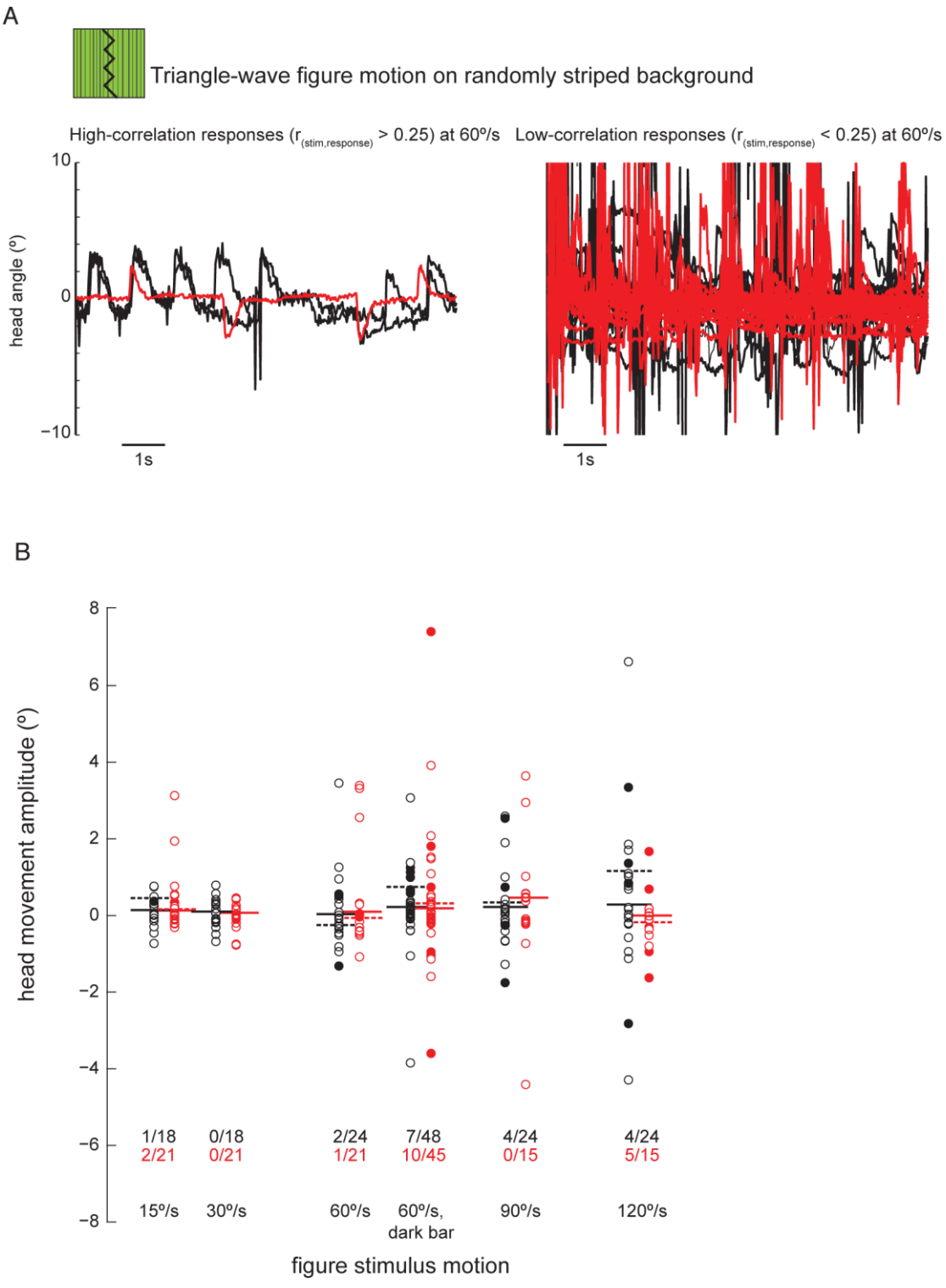

Figure 6. Haltere removal does not affect head movement responses to moving figures. A) High-correlation (left) and low-correlation (right) trials for intact (black) and haltereless (red) flies. Response amplitudes and correlation with the stimulus are generally low for all flies.

B) Response amplitudes of intact (black) and haltereless (red) flies at increasing angles of figure motion at a constant velocity. There were no significant differences between intact and haltereless flies at any visual speed, or with a visual stimulus of a dark bar on a bright background. As in Fig. 4, high-correlation trials ($|r| > 0.25$) are indicated by filled circles and low-correlation trials are indicated by open circles. Solid lines indicate the median amplitudes of all trials; dashed lines indicate the median amplitudes of high-correlation trials. Numbers below the data indicate the number of responsive trials as a fraction of the total number of trials. Trials with amplitudes above 8 and below -8° are included in the analysis but not shown (one intact fly at 15° sec^{-1}, one haltereless fly at 60° sec^{-1}, and one haltereless fly at 60° sec^{-1} in the dark bar condition).

Supplemental movies

Movie 1

Part 1: A fly standing on a glass coverslip and stimulated with wide-field visual motion. Non-flying flies do not show stabilizing gaze responses to triangle-wave wide-field motion, although they do show sporadic head movements.

Part 2: An intact fly suspended in the flight arena, but not flying, while stimulated with wide-field visual motion. Wingless flies do not show stabilizing gaze responses if they are not attempting to fly. "Flight" or "non-flight" states were determined by observing the fly's wings and halteres; this fly's legs are not retracted and its halteres are stationary.

Part 3. A wingless fly attempting to fly while stimulated with wide-field visual motion. Wingless flies show stabilizing gaze responses when they are attempting to fly. The moving (blurred) halteres and retracted forelegs indicate attempted flight.

Movie 2

Part 1. Intact and haltereless flies show a robust gaze stabilization response when stimulated with wide-field motion at 30° sec^{-1}. Left: intact fly; right: haltereless fly.

Part 2. Left: intact flies show a strong gaze stabilization response when stimulated with wide-field motion at 90° sec^{-1}. Note that the amplitude of head movement is maximal, with the head rotating until it touches the edge of the thorax. Right: haltereless flies show an obvious, but smaller, gaze stabilization response when stimulated with wide-field motion at 90° sec^{-1}.