

## RESEARCH ARTICLE

# Haltere mechanosensory influence on tethered flight behavior in *Drosophila*

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## ABSTRACT

In flies, mechanosensory information from modified hindwings known as halteres is combined with visual information for wing-steering behavior. Haltere input is necessary for free flight, making it difficult to study the effects of haltere ablation under natural flight conditions. We thus used tethered *Drosophila melanogaster* flies to examine the relationship between halteres and the visual system, using wide-field motion or moving figures as visual stimuli. Haltere input was altered by surgically decreasing its mass, or by removing it entirely. Haltere removal does not affect the flies' ability to flap or steer their wings, but it does increase the temporal frequency at which they modify their wingbeat amplitude. Reducing the haltere mass decreases the optomotor reflex response to wide-field motion, and removing the haltere entirely does not further decrease the response. Decreasing the mass does not attenuate the response to figure motion, but removing the entire haltere does attenuate the response. When flies are allowed to control a visual stimulus in closed-loop conditions, haltereless flies fixate figures with the same acuity as intact flies, but cannot stabilize a wide-field stimulus as accurately as intact flies can. These manipulations suggest that the haltere mass is influential in wide-field stabilization, but less so in figure tracking. In both figure and wide-field experiments, we observe responses to visual motion with and without halteres, indicating that during tethered flight, intact halteres are not strictly necessary for visually guided wing-steering responses. However, the haltere feedback loop may operate in a context-dependent way to modulate responses to visual motion.

**KEY WORDS:** Halteres, Multimodal sensing, Vision, Flight control

## INTRODUCTION

When flying, the stability of flies is maintained in part by the integration of sensory information from reduced hindwings known as halteres. Halteres retain many of the cuticular mechanosensors that are present on the hindwings of other insects, but they are much shorter than hindwings and dumbbell-shaped, with a bulb at their distal tip. Halteres provide a purely sensory function and do not generate significant lift. The haltere base is equipped with several fields of strain-sensitive campaniform sensilla (~300 in *Drosophila*), a small number of sensory hairs and a chordotonal organ. The campaniform sensilla impart sensory information about the angular velocity of body rotations (in blowflies: Nalbach, 1993; in fruit flies: Dickinson, 1999) and also provide timing signals to the steering muscles of the wings (in blowflies: Heide, 1983; Fayyazuddin and Dickinson, 1999). Haltere movement stimulates the campaniform sensilla (Pringle, 1948; Fox and Daniel, 2008) and

this information is transmitted to wing-steering motoneurons (Fayyazuddin and Dickinson, 1996), gaze control motoneurons (Sandeman and Markl, 1980; Huston and Krapp, 2009), and other locations in the nervous system (Chan and Dickinson, 1996).

An important function of the haltere is to detect involuntary body rotations, send this information to the wing-steering muscles and adjust steering to compensate for such perturbations (Hengstenberg, 1991; Nalbach, 1993; Dickinson, 1999; Sherman and Dickinson, 2003). In this way, the haltere reflex causes rapid changes in wing steering to maintain stability. A central problem with these reflexes, however, is that such a system would not permit the fly to make a voluntary turn. If the fly intentionally turned its body, the same forces would occur on the haltere as would occur during an involuntary turn, and the haltere neurons would signal to the wing-steering complex to return the fly to its original course. Therefore, the haltere reflex must be modulated in some way to distinguish self-generated from externally generated turns and permit voluntary movement (von Holst and Mittelstaedt, 1950).

A potential mechanism for differentiating voluntary turns from involuntary turns was proposed by Chan et al. (1998). When recording activity in the haltere and wing muscles of a dissected blowfly while presenting a visual stimulus, they found that muscles controlling haltere motion, but not muscles controlling wing steering, were modulated by visual input. This link between the visual system and the haltere muscle presents a means by which the visual system could 'tune' the haltere's motion, modifying the sensory input from the haltere pathway.

If the visual system can exert control over the haltere's trajectory, there are at least two ways the fly could use this control to execute a voluntary turn. First, the visual system could simply inhibit signals from the haltere at any point along the sensory-motor pathway, perhaps using inhibitory interneurons to reduce the nervous system's response to haltere movements. In this scenario ('reflex desensitization'), a copy of the expected haltere input resulting from the intended motor activity would be sent to the haltere sensory pathway and decrease responses to the anticipated haltere input. This signal associated with the efference copy could be a graded response, specific to the expected input (as seen in the cerebellum-like structure of electric fish; Bell, 1981), or it could be a complete block of the neural response via inhibitory interneurons (as seen in the auditory system of the singing cricket; Poulet and Hedwig, 2002). During this 'reflex desensitization', however, flies would be unable to distinguish self-generated turns from perturbations, making them vulnerable to instability.

Alternatively, the fly could actively adjust the halteres' movements to mimic those experienced during external perturbations and, in doing so, manipulate the haltere to drive a corrective response in the intended trajectory. In the absence of external perturbation, this corrective response, driven by active motions of the haltere, would result in a voluntary turn. This is the 'control-loop manipulation' hypothesis (Chan et al., 1998). In this

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scenario, wing-steering controls are driven entirely by sensory input from the halteres, which themselves receive descending commands to their muscles from the visual system.

This hypothesis presents a question: given that visual motion does not induce supra-threshold responses in wing-steering muscles to visual motion (Chan et al., 1998), does the wing-steering response to visual motion then require input from the haltere? In other words, does the haltere take on the task of informing the wing-steering muscles about visual motion, and therefore drive optomotor (and perhaps other) wing-steering responses? The control-loop manipulation hypothesis suggests that flies without halteres, or with the mass on their halteres ablated to decrease the force on the campaniform sensilla at the base, would be unable to execute voluntary turns.

Because flies without halteres cannot initiate free flight, it is difficult to assess the impact of halteres on flight behavior using classical sensory ablation methods. Haltereless flies will fly if tethered to a rigid pin, however (Dickinson, 1999), and by doing this, we were able to quantitatively examine the influence of haltere input on visually guided flight behavior. This artificial environment did not permit the fly to rotate, removing the confounding influence of forces generated by body rotations, and allowed us to observe the fly's behavior when it was experiencing only self-generated haltere movements in combination with visual stimuli. We tested the fly's steering responses to two distinct visual stimuli that drive distinct behavioral responses (Fox et al., 2014): a wide-field stimulus that drives the involuntary optomotor stabilization reflex and a moving vertical stripe that elicits voluntary figure-tracking behavior. Does the haltere influence these two behaviors in different ways?

The haltere is a multi-functional sensory organ, detecting both the Coriolis forces that result from body rotations (Pringle, 1948; Nalbach, 1993; Dickinson, 1999; Thompson et al., 2009) and the large inertial force that results from its oscillations in flight (Heide, 1983; Fayyazuddin and Dickinson, 1999). The Coriolis force is small relative to the flapping force (Thompson et al., 2009) and if the distal mass of the haltere is removed (Fig. 1B), the Coriolis force is even smaller (Nalbach, 1993; Sane et al., 2007). A fly on a rigid tether does not experience Coriolis forces resulting from attempted turns, as it would in free flight, but the haltere mass would be essential to the active haltere deflections that mimic Coriolis forces proposed by the control-loop manipulation hypothesis. Although neural recordings from haltere primary afferents or their neural targets have not been performed under these conditions, we designed our experiments under the assumption that flies with bulb-ablated halteres can no longer measure Coriolis forces, but are still able to experience signals related to the haltere beating cycle (the large inertial force). In contrast, flies with fully ablated halteres experience neither Coriolis forces nor any forces related to the haltere beating cycle. We observed the flies' behavioral responses to visual stimuli under both conditions to determine how each of these forces and their mechanosensory encoding might interact with visual motion information to structure flight behavior.

## RESULTS

### Haltere manipulations have small effects on wingbeat frequency and steering amplitudes

Flies were tethered to pins and placed above a photodiode that converts the wing's shadow, and thus its stroke amplitude, into a voltage, as described previously (Fig. 1A) (Dickinson, 1999; Reiser and Dickinson, 2010; Fox et al., 2014). We examined behavior in intact control flies, in flies with the haltere bulb ablated and in flies with the entire haltere, including campaniform sensilla, ablated

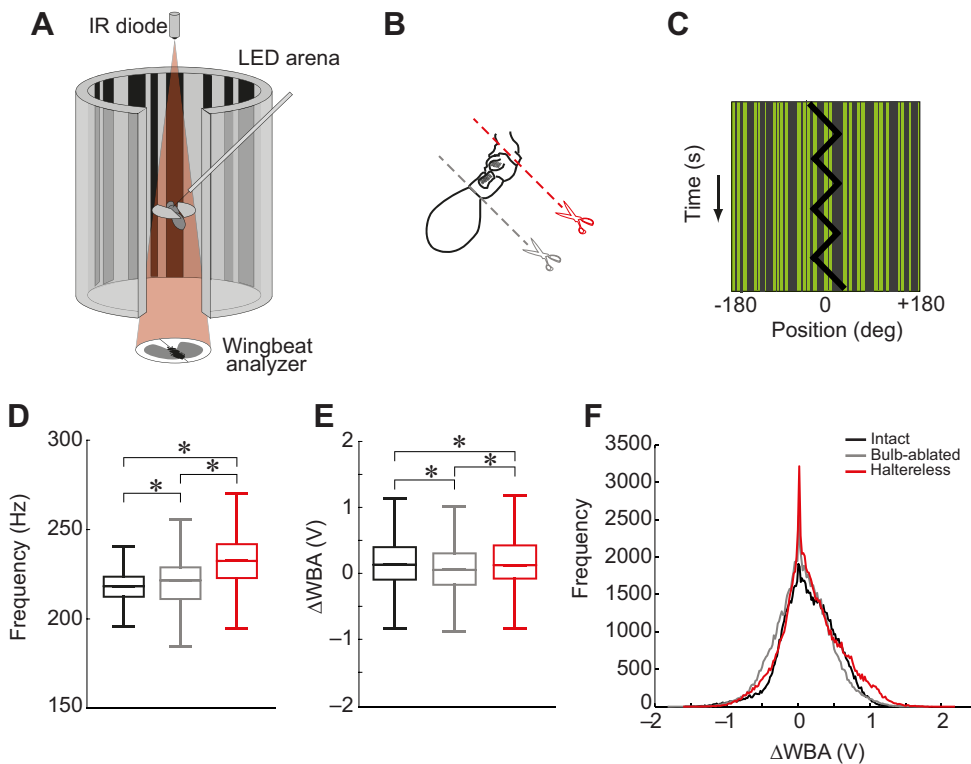
(Fig. 1B). Our stimulus patterns were displayed on an LED arena surrounding the fly. In these patterns, each row was identical and the vertical stripes comprising the wide-field and figure components of the pattern could be moved independently. The patterns (visual stimuli) are moved alternately in the clockwise and counter-clockwise direction at constant velocity. Such a stimulus function may be described as a triangle-wave function in the space–time domain. We show simplified schematics of the space–time plots of these stimuli in each of our figures (example in Fig. 1C).

In a previous report, tethered flies with one or both haltere bulbs ablated showed a 24% increase in wingbeat frequency (Dickinson, 1999). We examined the wingbeat frequency of all flies over all of our open-loop experiments ( $N=90,200$  sample points) and found that although the wingbeat frequency was significantly higher for bulb-ablated and haltereless flies (Kruskal–Wallis test,  $P<0.001$ ), these differences in our sample were very small: 1.3% higher for bulb-ablated flies and 6.4% higher for haltereless flies (median wingbeat frequencies for control, bulb-ablated and haltereless flies were 218, 221 and 232 Hz, respectively; Fig. 1D).

It is possible that haltere manipulations would render the flies unable to steer their wings with the same amplitudes as their intact counterparts. We performed these experiments using an uncalibrated instrument – a photodiode-based wingbeat analyzer – and thus we can only comment on the relative amplitudes and amplitude modulations, not on the absolute amplitudes of the flies' wingbeats. We measured the difference in amplitude between the two wings for all of our open-loop experiments and plotted their distribution. As in the wingbeat frequency measurements, we found statistically significant but numerically very small differences in the median of wing-steering amplitudes between intact flies and flies with the bulb or entire haltere ablated ( $N=90,200$  sample points). This indicates that the haltereless, bulb-ablated and intact flies have similar median steering and there is no offset in wing amplitudes caused by haltere manipulation (median wing amplitudes as measured by the photodiode for control, bulb-ablated and haltereless flies were 0.13 V, 0.05 V and 0.12 V, respectively, Fig. 1E). More importantly, the flies did not show any significant difference in the variances of their wing steering, indicating that the range of steering efforts was not altered by haltere manipulation (Fig. 1F, two-sample  $F$ -test for equal variances,  $P>0.2$ ). Thus, haltere-manipulated flies are capable of, and do show, the same amount of relative wing-steering effort as the intact control flies.

### Haltereless flies rapidly fluctuate their wing amplitudes

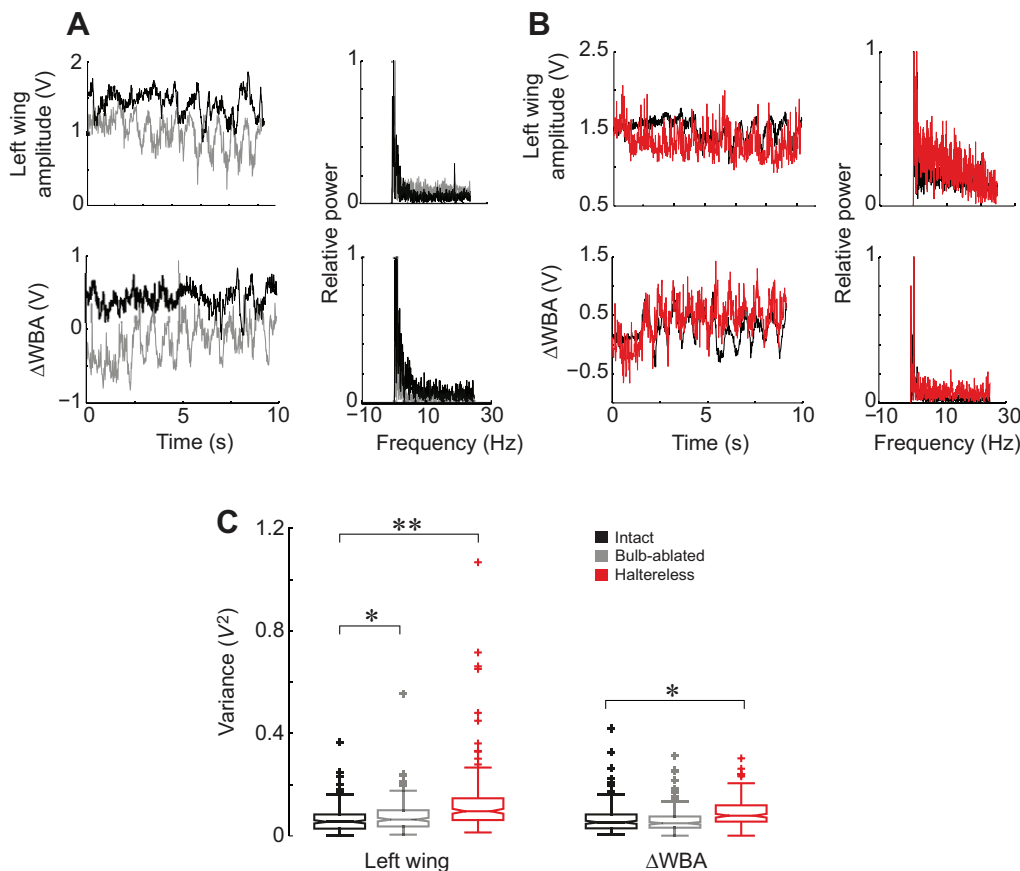
We noted during our experiments that the voltage traces from the photodiode, reflecting the fly's wing strokes, were visibly more variable for haltereless flies than for intact or bulb-ablated flies, with large fluctuations in peak amplitude on each stroke. How is this reflected in our data and could it prevent haltereless flies from steering towards a visual stimulus? We examined the wing amplitude traces from the wingbeat analyzer for each fly during all of our open-loop wide-field stimulation experiments and noted that the steering metric of interest, the difference of left and right wingbeat amplitudes, was more variable in haltereless flies than intact flies (Fig. 2B, bottom). This variability was less apparent in bulb-ablated flies (Fig. 2A, bottom). Is this variability due to rapid fluctuations in steering effort, rapid fluctuations in the amplitudes of the wings independently, or some combination of the two? Fourier transforms of single wingbeat amplitude traces (left or right wing only) indicate that haltereless fly wingbeat amplitudes show more relative power at higher frequencies than intact fly wingbeat



**Fig. 1. Haltere manipulations in *Drosophila* have minor effects on wingbeat frequency and steering amplitude.** (A) Schematic of the flight arena. (B) Bulb removal left campaniform sensilla intact (gray); full removal ablated campaniform sensilla (red). In all subsequent figures, data from bulb-ablated flies is shown in gray, and data from haltereless flies is shown in red. (C) Schematic of a space-time plot depicting pattern motion for one of our experiments. In this case, a figure (a vertical black bar) is moved in open-loop (OL) (alternating clockwise and counterclockwise motion constant angular velocities in a 'triangle wave' function) against a static background. Schematics of stimuli follow this convention in subsequent figures. (D) Boxplot of wingbeat frequencies for intact, bulb-ablated and haltereless flies ( $N=90,200$  samples for each group). (E) Boxplot of wing-steering effort ( $\Delta WBA$ ) for the three haltere manipulations ( $N=90,200$  samples for each group). (F) Histogram of wing-steering effort (same data as in E). The medians of the three groups were statistically significantly different from each other in D and E, as shown (Kruskal–Wallis test,  $*P<0.001$ ).

amplitudes (Fig. 2B), which is not the case for bulb-ablated flies (Fig. 2A). We also found that the variances of single wing amplitudes, as measured over 10 s trials in our open-loop experiments, were significantly higher in bulb-ablated and

haltereless flies than in intact flies (Kruskal–Wallis test,  $P<0.05$  for bulb-ablated flies,  $P<0.01$  for haltereless flies;  $N=200$  trials for each group; Fig. 2C, left). However, this difference was smaller (and not significant in the case of bulb-ablated flies) when examining the



**Fig. 2. Haltereless flies have more-variable single-wing amplitude traces than intact flies, but the difference between two wingbeat amplitudes is not significantly more variable.** (A) Comparison of a single trial in one bulb-ablated fly (gray) to a single trial in one intact fly (black). Top left: left wingbeat amplitude in response to moving wide-field stimulus. Top right: Fourier transform of traces to the left. Bottom:  $\Delta WBA$  response and associated Fourier transform for the same trial shown in traces above. (B) Single-trial comparisons for haltereless (red) and intact flies (black). (C) Comparison of variances in left wingbeat amplitude and  $\Delta WBA$ ,  $N=20$  flies. The boxplots here and in other figures show the 25th and 75th percentiles (top and bottom of the box), the median (line in the middle of the box), data range (whiskers), 95% confidence intervals (notches) and outliers (+ symbols). Variance in left wingbeat amplitude was different in haltereless flies when compared with intact and bulb-ablated flies (Kruskal–Wallace test,  $*P<0.05$ ,  $**P<0.01$ ). Haltereless flies had significantly larger variance in  $\Delta WBA$  when compared with intact flies, but not bulb-ablated flies. Analysis of left wing is shown here; the analysis of right wing data showed the same results.

variances in  $\Delta WBA$  (Fig. 2C, right). Fourier transforms of the  $\Delta WBA$  show less relative power at the high frequencies than the Fourier transforms of single wing amplitudes. This suggests that haltereless flies change their wing stroke amplitudes much more frequently than intact flies, but that the two wings do so roughly in synchrony such that the difference between the two does not change as frequently. Thus, although wing control is more variable in bulb-ablated and haltereless flies, wing coordination in general is not.

### Haltere bulb ablation decreases responses to wide-field visual motion but not figure motion

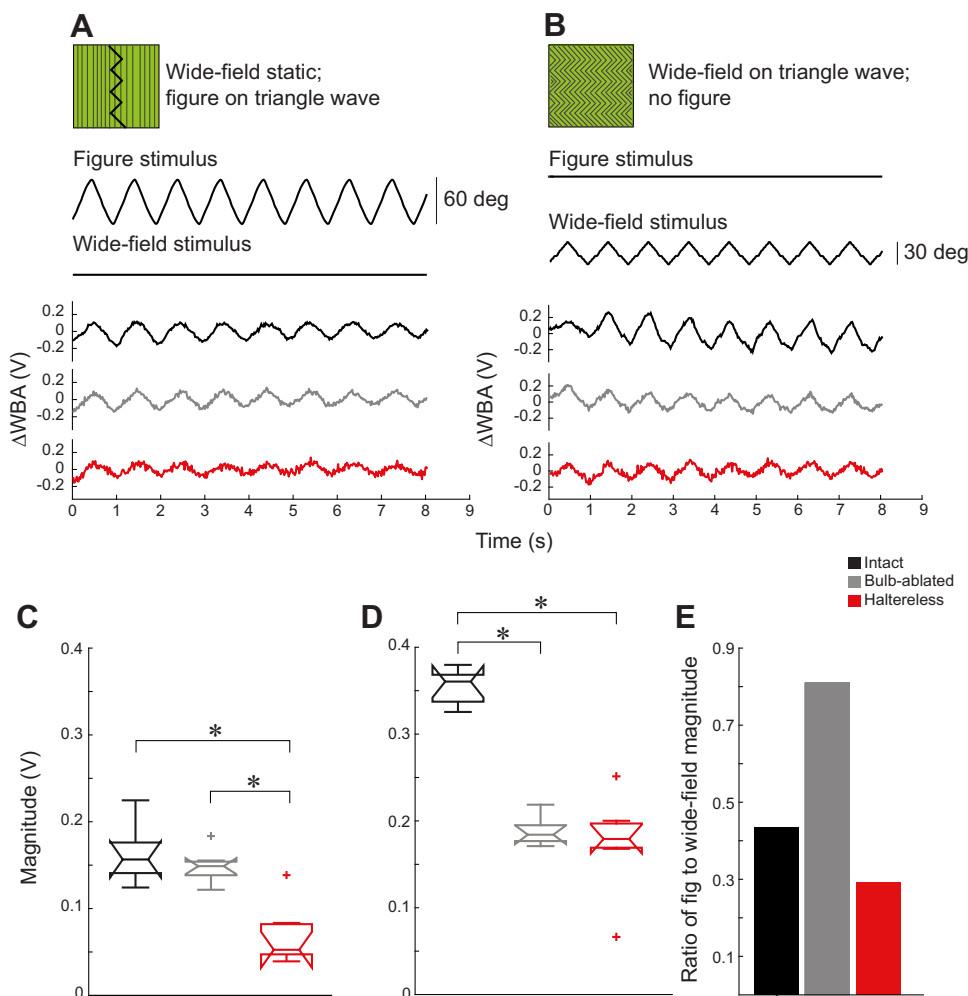
How does the removal of the haltere mass or of the entire haltere affect the fly's responses to visual stimuli? Using triangle-wave functions as described above, either a figure was moved against a stationary background, or the wide-field background pattern was moved. We found that removing the haltere bulb did not decrease the response to figure motion, but removing the entire haltere did decrease the response (Fig. 3A,C). In contrast, when we oscillated the wide-field pattern only, the response of bulb-ablated flies was significantly reduced, and removing the entire haltere did not reduce it further (Fig. 3B,D). The same groups of flies were used for both wide-field and figure motion experiments, and we noted that the response of the haltereless flies to the wide-field stimulus was similar in magnitude to the intact flies' response to figure motion. Thus, we know that the haltereless flies were physically capable of producing the same magnitude of wing steering as the intact flies because we observed them doing so in the wide-field experiment.

This indicates that the smaller magnitude response observed here is neither a result of impaired flight, nor a simple mathematical consequence of the increased variability in wingbeat amplitude resulting in a decrease in the magnitude of the mean response. Each stimulus was manipulated to produce the largest possible response, resulting in a figure motion that was twice the extent and speed of the wide-field motion, but the figure response was still approximately half the magnitude of the wide-field response for intact flies. We noted that the ratio of the figure response magnitude to wide-field response magnitude was approximately 1:2 for intact and haltereless flies, but the two responses were more equal for bulb-ablated flies (Fig. 3E). In all cases, the fly's response to the visual motion is still discernable and is phase-locked to the periodic stimulus, and we did not observe any changes other than a decrease in the magnitude of the response in haltere-manipulated flies.

### The ability of haltereless flies to stabilize a wide-field pattern in closed loop is decreased, especially at high gains

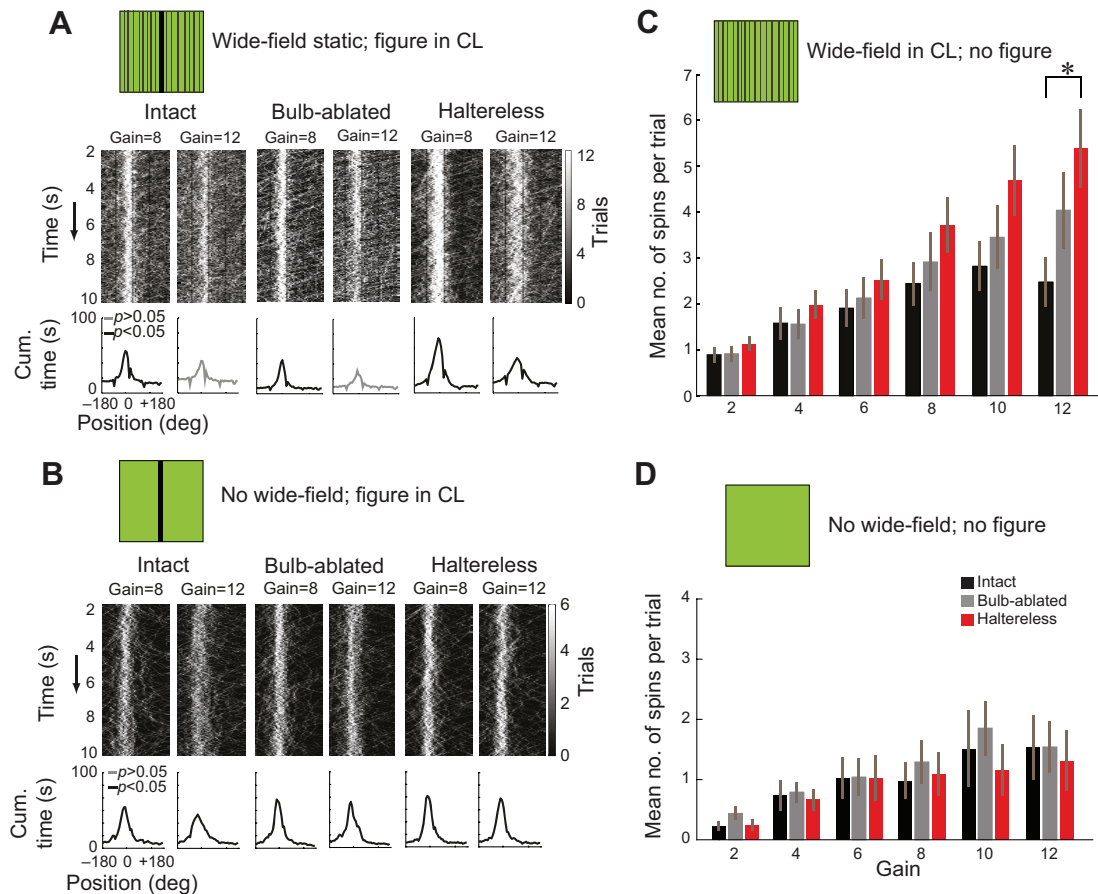
We closed the feedback loop between steering efforts (as measured by the wingbeat analyzer) and angular velocity of the visual stimulus to examine the flies' ability to stabilize their visual surroundings. We tested their performance when stabilizing a moving figure against a static pattern and a moving wide-field pattern (as above).

When stabilizing a figure in the frontal field of view, intact, bulb-ablated and haltereless flies showed similar performance at varying gains, as measured by vector strength calculations (Fig. 4A).



**Fig. 3. Comparison of data obtained from intact, bulb-ablated and haltereless flies in open-loop conditions.** (A) Mean responses to moving figure in open loop (OL) on static wide-field pattern. (B) Mean responses to moving wide-field pattern in OL with no figure. (C) Boxplots of response magnitude for the three groups, with significant differences observed in haltereless flies in comparison to intact and bulb-ablated flies (Wilcoxon rank-sum test,  $*P < 0.05$ ). (D) Boxplots of response magnitudes for the three groups, with significant difference observed for bulb-ablated and haltereless flies in comparison to intact flies (Wilcoxon rank-sum test,  $*P < 0.05$ ). (E) Comparison of the ratios of median figure response to median wide-field response.  $N = 100$  trials for all groups (20 flies, 5 trials per fly).





**Fig. 4. Comparison of data obtained from intact, bulb-ablated and haltereless flies in closed-loop conditions.** (A) Traces of figure position in closed loop (CL) with static wide-field background for all flies. Histograms show cumulative time at each arena position. Figure position distributions that are significantly different from uniform are shown in black (Rayleigh z-test,  $*P < 0.05$ ), not significant in gray. All groups of flies (intact,  $N = 25$  flies; bulb-ablated,  $N = 24$  flies; haltereless,  $N = 25$  flies; 3 trials per fly) showed statistically significant figure position distributions at lower gains (not shown). (B) Traces of figure position in CL with no visible wide-field pattern. Data as in A ( $N = 20$  flies in all groups, 3 trials per fly). (C) Spin counts for wide-field pattern motion at six gains. Haltereless flies show more spins than intact and bulb-ablated flies at all gains, and significantly more than intact flies at the highest gain (Wilcoxon rank-sum test,  $*P < 0.05$ ;  $N = 31$  flies, 3 trials per fly for all groups). (D) Spin counts for all groups of flies with no visible wide-field pattern (intact,  $N = 15$  flies; bulb-ablated,  $N = 16$  flies; haltereless,  $N = 15$  flies; 3 trials per fly). No significant differences were observed between groups. Error bars in C and D show s.e.m.

Surprisingly, haltereless flies showed slightly better performance at high gains than intact flies. These results indicate that figure-tracking ability in closed loop is not affected by haltere manipulations. In a control experiment, in which we removed the static random stripes from the wide-field pattern and presented a dark bar against a bright background, all groups of flies were able to fixate the bar at all gains. However, haltereless flies did so with markedly higher precision than intact or bulb-ablated flies, showing a much sharper peak in the position histogram at the highest gain (Fig. 4B).

In contrast, haltereless flies were less able to stabilize a wide-field pattern. Because vector strength is a position-specific metric and the wide-field pattern, by design, does not contain position-specific information, we used a different metric for stability when considering wide-field motion responses. If the fly loses control of the pattern, it will spin rapidly around the arena. We therefore counted the number of complete rotations ('spins') of the pattern around the arena in a given trial and used this as an inverse metric of the flies' ability to stabilize the pattern. Of several metrics considered, including maximum velocity, average velocity and position variance, spin count most accurately and intuitively captured the behavior we observed. This count is necessarily influenced by gain, since rotation velocity is higher at higher gains,

and thus we see more spins in all groups of flies as gain increases. At all of the gains studied, the mean spin count was higher for haltereless flies than for intact and bulb-ablated flies, and this spin count was significantly higher at the highest gain (Fig. 4C; Wilcoxon rank-sum test,  $P < 0.05$ ). Although bulb-ablated flies exhibit higher spin counts than intact flies across all gains, the difference was not significant.

Could these results be explained by a general increase in steering instability in the haltereless flies, perhaps related to the higher variability observed in the raw data (Fig. 2)? If the haltereless flies showed erratic steering behaviors regardless of the visual stimulus, the spin count might be artificially high, and not informative about the flies' response to visual stimulation. We therefore performed a control experiment to examine the baseline rate of pattern spinning in the absence of visual stimuli. We replaced the randomly striped wide-field pattern with a pattern in which all of the LEDs were turned on or off and repeated the wide-field stabilization experiment as above. In this experiment, the pattern can still rotate around the arena as a result of the fly's wing steering (and such rotations are recorded), but since all of the LEDs are on (or off), there is no visible result of wing-steering efforts. In this way, we could monitor the number of spins that are due to the fly's steering behavior in the absence of visual stimulation. We found that the spin counts of

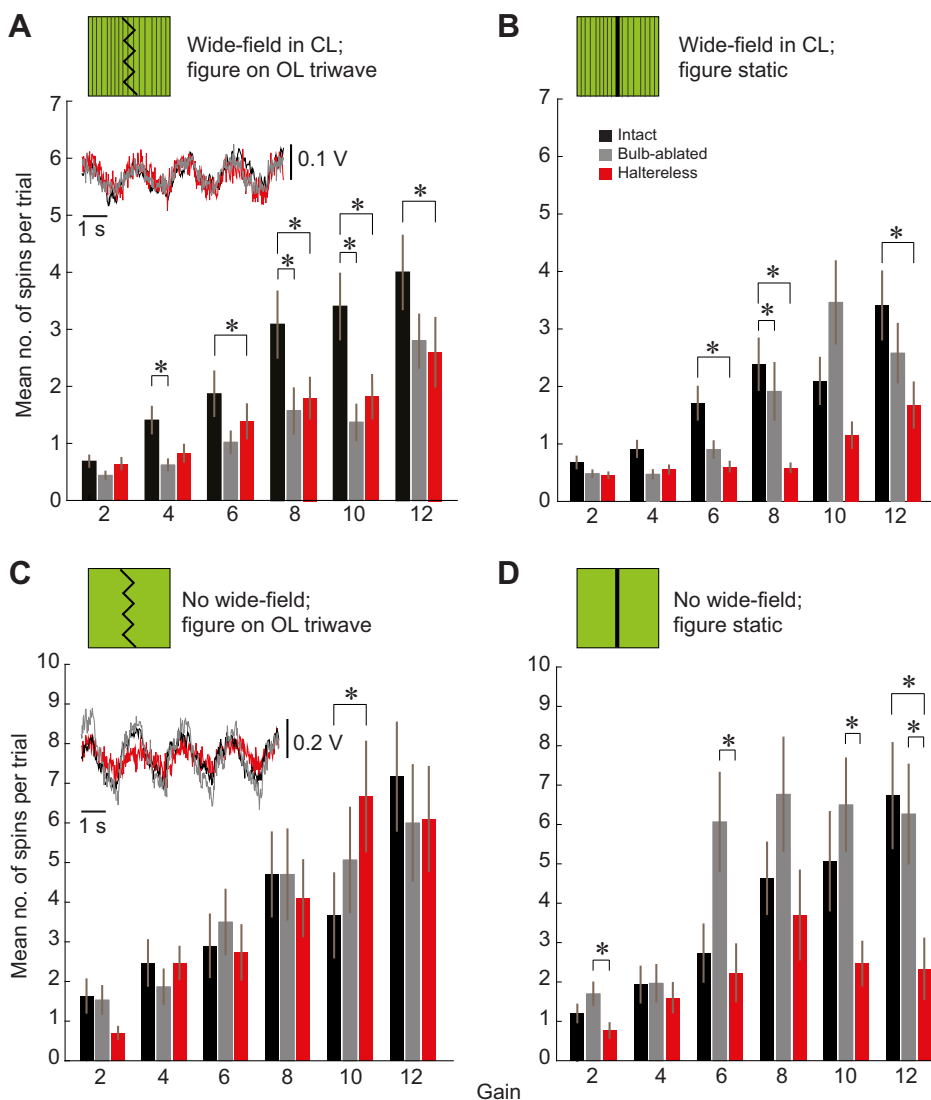
intact, bulb-ablated and haltereless flies were not different under these conditions, indicating that the differences in wide-field pattern stabilization between experimental groups are due to differences in responses to the visual stimulus rather than differences in baseline steering behavior (Fig. 4D).

### Figure tracking improves wide-field stabilization behavior in haltereless flies

We further examined closed-loop behavior by placing a figure in the arena in addition to the closed-loop wide-field pattern. First, this figure was moved back and forth in open loop, using the same triangle-wave function used in the open-loop experiments described above (Fig. 5A). As above, the wide-field panorama was in closed loop with variable gains. We found that haltereless flies performed better, with significantly lower spin counts than intact flies at higher gains (Fig. 5A). Bulb-ablated flies generally performed better than intact flies across all gains, but statistically significant differences in the spin counts were observed only at three of the gains tested (gain=4,  $P=0.02$ ; gain=8,  $P=0.001$ ; gain=10,  $P=0.002$ ). Intact, bulb-ablated and haltereless flies showed similar magnitudes of wing-steering responses to the moving figure (Fig. 5A, inset). Next, we kept the figure static in the front of the arena. We observed that haltereless flies were much better able to stabilize the pattern than

intact flies, with significantly fewer pattern spins, especially at high gains (Fig. 5B). Bulb-ablated flies had lower spin counts than intact flies at each gain, and this difference was significant at one of the gains tested (gain=8,  $P=0.03$ ).

How much of this effect can be explained by the fly's response to the figure alone, and how much is explained by the interaction of the figure and the wide-field stimulus? To answer this, we performed a control experiment wherein we removed the random stripes from the wide-field pattern and placed a dark moving (Fig. 5C) or static figure (Fig. 5D) in the center of the arena, similar to the experiment shown in Fig. 4D. We observed that all three groups of flies had higher spin counts across all gains compared with the experiment using wide-field motion (Fig. 5A), with the haltereless flies performing significantly worse than intact flies at one of the higher gains tested (gain=10,  $P=0.03$ ). In addition to counting the spins of the wide-field pattern, we measured the wing-steering responses of the flies to the moving figure. The magnitude of the wing-steering response of intact and bulb-ablated flies to a moving figure was about twice as large as the response observed with a visible wide-field stimulus, but responses of haltereless flies were smaller (Fig. 5C, inset), consistent with results above (Fig. 3A,C). When a static figure was placed in a bright LED arena in a similar experiment, spin counts for all the three groups were higher



**Fig. 5. A static or moving figure in the frontal field of view improves stabilization behavior in haltereless flies.** (A) When a figure moves in OL against a wide-field pattern in CL, bulb-ablated and haltereless flies show fewer spins (Wilcoxon rank-sum test,  $*P<0.05$ ;  $N=31$  flies in each group, 3 trials per fly), and similar wingbeat responses compared with intact flies (inset). (B) When a static figure is placed in front of the fly against a wide-field stimulus in CL, bulb-ablated flies ( $N=31$  flies) perform slightly better than intact flies ( $N=33$  flies) at all gains, and haltereless flies ( $N=33$  flies) show significantly fewer spins of the pattern than intact flies at three tested gains (Wilcoxon rank-sum test,  $*P<0.05$ ). (C,D) Control experiments for A and B, in which the three groups of flies ( $N=10$  for all groups) tracked a dark moving or static figure in OL on a bright field in CL. In the absence of a visible wide-field pattern, all groups of flies show higher spin counts in general. Haltereless flies performed significantly worse than intact flies at gain=10 (Wilcoxon rank-sum test,  $*P<0.05$ ) when the figure was moving, and significantly better than intact and bulb-ablated flies at higher gains tested when the figure was static (Wilcoxon rank-sum test,  $*P<0.05$ ). All error bars indicate s.e.m.

compared with the experiment with visible wide-field motion (Fig. 5D). Although bulb-ablated flies showed higher spin counts than intact flies, their performance was not statistically different from that of intact flies. Haltereless flies generally performed better, with lower spin counts compared with intact and bulb-ablated flies, but a statistically significant difference was only observed at the highest gain tested (gain 12,  $P=0.01$ ) when compared with intact flies and four of the gains tested (gain=2,  $P=0.02$ ; gain=6,  $P=0.02$ ; gain=10,  $P=0.009$ ; gain=12,  $P=0.008$ ) when compared with bulb-ablated flies. Taken together, these results indicate that haltereless flies are better able to stabilize a wide-field pattern when they are also tracking a figure, and that the increase in stability is most pronounced in the presence of wide-field motion.

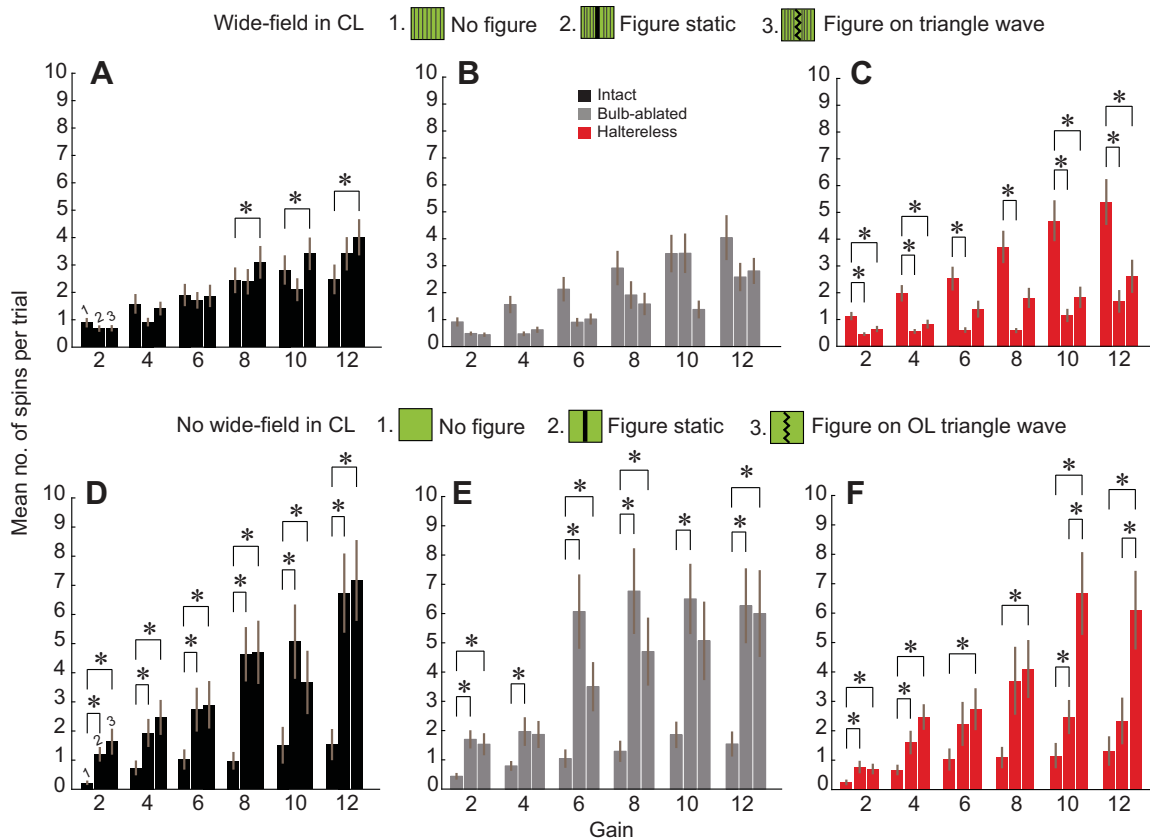
### The presence of a figure helps haltereless flies stabilize a wide-field pattern

To examine the overall effects of the various stimuli on the three treatment groups, we re-plotted the data from Figs 4 and 5 to compare spin counts within a single group. For intact flies, the presence of a figure against a closed-loop wide-field pattern only increases spin count at high gains, and only if the figure is moving (Fig. 6A); in general, the presence of a figure does not have a particularly strong effect on wide-field stabilization in intact flies. Similarly, for bulb-ablated flies, there is no effect of a figure on the spin count (Fig. 6B). In haltereless flies, the presence of a static figure significantly decreases the spin count at all gains, and the presence of a moving figure decreases the spin count at some gains

(although the spin count for a moving figure was always higher than the count for a static figure; Fig. 6C). To determine the effect of the wide-field background on the flies' behavior, we performed the same experiment with a uniform background (no visible pattern). Here, intact and haltereless experienced similar effects: the presence of a static figure increased the spin count at each gain, and a moving figure increased it even further. For bulb-ablated flies, however, the spin count was lower with a moving figure than with a static figure. In each group, the responses to moving figures with and without wide-field closed-loop stimulation were different, suggesting complex interactions between figure tracking and optomotor responses. The 'rescue' of haltereless fly responses by the static bar, with a lower spin count as compared with both intact flies (Fig. 5B,D) and the haltereless flies' responses to a wide-field stimulus with no figure (Fig. 6C), is most pronounced when the wide-field background pattern is present. This suggests that the 'rescue' phenomenon is the result of the interaction between wide-field and figure-tracking responses and not a result of the figure-tracking response alone.

### DISCUSSION

By tethering flies to pins, we are able to examine how flies process visual stimuli in the absence of halteres, and in the absence of the body rotations that halteres are known to sense. Tethering the flies provides artificial stability to the fly, unlinking the fly's steering maneuvers from actual body rotations and thus, from the expected haltere feedback due to Coriolis forces. Haltere muscles may be



**Fig. 6. Compilation of data comparing effects of figure and wide-field interaction within intact, bulb-ablated and haltereless flies.** (A–C) Spin counts for intact, bulb-ablated and haltereless flies ( $N=31$  flies, 3 trials per fly) stabilizing a wide-field pattern in CL with no figure, with a static figure and with a moving (triangle-wave function) figure in OL at various gains (Wilcoxon rank-sum test,  $*P<0.05$ ). (D–F) Control experiments for A–C with no visible wide-field pattern (Wilcoxon rank-sum test,  $*P<0.05$ ). For no wide-field and no figure experiment: intact,  $N=15$ ; bulb-ablated,  $N=16$ ; haltereless,  $N=15$  flies, 3 trials per fly. For all other experiments,  $N=10$  flies for all groups. All error bars indicate s.e.m.

actively modulated by the visual system (Chan et al., 1998), and we were able to test the hypothesis that halteres influence visually guided steering behavior even in the absence of body rotations. We found that the effects of haltere ablation are more profound for wide-field motion tasks than for figure-tracking behavior in open loop, but that haltereless flies can still track all visual motion stimuli. In closed loop, haltereless flies are less able to stabilize a wide-field stimulus, but can track figures as well as, or better than, intact flies.

### **The control-loop manipulation hypothesis explains some, but not all, of the tethered flight behavior of haltereless flies**

Our results indicate that flies without the haltere mass do not show the full magnitude of response when presented with wide-field motion (Fig. 3D). However, the haltere mass is not necessary for flies to respond normally to moving figures (Fig. 3C). The figure response measured here was always smaller than the wide-field response, even when the amplitude of the figure's movement was increased. Could our results be explained by a general inability of the bulb-ablated flies to steer their wings with the same amplitude difference seen in intact flies? Our data suggest not. The bulb-ablated flies, as well as the haltereless flies, show the same range of responses as intact flies (Fig. 1D,E) suggesting that their overall wing-steering abilities are not affected by the haltere manipulations. Haltereless flies show increased amplitude variance in single wings, but less of an increase in variance is observed in the difference between the two wings (Fig. 2C). Furthermore, haltereless flies are able to produce the same magnitude of response to wide-field motion as intact flies do to figure motion (Fig. 3).

The persistence of the response to visual stimuli in the face of moderate and severe haltere manipulations indicates that there must be a haltere-independent connection between the visual system and steering muscles of the flight motor in *Drosophila*. Although the wide-field steering response is significantly diminished by the loss of the haltere bulb or the entire haltere, the response is clearly still present, even in flies with the entire haltere ablated. This is also the case for figure tracking responses, which are generally smaller than the wide-field responses.

Taken together, our results show that the halteres are important for full-amplitude wing-steering responses to visual motion, especially wide-field motion. However, we show that they are not an essential component of the circuit that permits the wings to steer the fly towards a visual stimulus. The control-loop manipulation hypothesis was proposed based on recordings from haltere and wing-steering muscles in dissected blowflies. Under those conditions, visual stimuli did not evoke supra-threshold responses in wing-steering muscles as they did in haltere muscles (Chan et al., 1998). The authors of that study raise the possibility that neural gating of visual information may vary depending on locomotor state, a phenomenon observed and characterized later in the visual system of flies (Longden and Krapp, 2009; Chiappe et al., 2010; Maimon et al., 2010; Suver et al., 2012). Furthermore, the experiments leading to the control-loop manipulation hypothesis were performed on blowflies, which diverged from fruit flies approximately 50 million years ago (Hedges and Kumar, 2009) and may have different haltere-related circuits from those in fruit flies. In addition to their distinct evolutionary paths, *Drosophila* and *Calliphora* are of considerably different sizes and experience different aerodynamic and inertial forces in flight, which may require different neuromuscular activity for stability and control in flight. The haltere-related behavioral differences between the species are largely unexplored, but there may be fundamental disparities in circuit function that explain our observations here.

### **Why can't flies fly without their halteres?**

It has been known for hundreds of years that flies without their halteres are unable to fly (Derham, 1714). What, precisely, are they missing when their halteres are removed? We show here that flies without the bulb and without their entire haltere can still respond to visual motion stimuli, both small figure motion and wide-field motion (Fig. 3). Thus, the inability of haltereless flies to take flight cannot be explained by a lack of visual input to the wing-steering complex.

The noisy raw traces of the amplitudes of single wings suggest that haltereless flies are less able to control their wing amplitudes, even though the steering response ( $\Delta WBA$ ) does not appear to be strongly affected by haltere removal (Figs 1 and 2). The increased variance in single-wing amplitude traces in haltereless flies (Fig. 2C) is consistent with prior observations that the haltere oscillation provides synchronizing input to the wing-steering motor neurons (Fayyazuddin and Dickinson, 1999). Recent work indicates that for flies in free flight, only very small wing amplitude changes (1–3 deg) are required to rotate the body into evasive maneuvers (Muijres et al., 2014; Schnell et al., 2014). The higher wingbeat amplitude traces of individual haltereless flies suggest that even if flies are able to respond to visual stimuli without their halteres, their small high-frequency changes in wingbeat amplitude (Fig. 2B) could lead to catastrophic instability in free flight. This instability may explain why haltereless flies are unable to fly, and may also explain how our tethered preparation allowed us to stabilize the fly and thus observe the haltere's influence on visual behavior.

Although the high-frequency amplitude changes in haltereless flies may explain their inability to fly, we also observed that bulb-ablated flies, which do not show the same increase in variance in their wing amplitudes, could not initiate free flight. Flies without their haltere bulb experience substantially reduced Coriolis forces during body rotations (Nalbach, 1993; Sane et al., 2007) and show a significantly reduced response to visual wide-field motion (Fig. 3). Thus, the inability of both haltereless and bulb-ablated flies to take flight testifies to the necessity of both of the haltere's functions: it suggests that the bulb is needed for freely moving flies to integrate body rotation information, and that the campaniform sensilla at the base are needed for precise timing input to the wing-steering motoneurons (Heide, 1983; Fayyazuddin and Dickinson, 1996, 1999).

### **Haltere influence on figure and wide-field tracking**

Our results show that although the halteres are not essential for processing visual information, they are involved in structuring the response to visual stimuli and do so in a context-dependent manner. Removing the halteres decreases the flies' ability to stabilize a wide-field pattern in closed loop (Fig. 4C), but does not decrease their ability to stabilize a moving figure (Fig. 4A); rather, it slightly increases their figure-tracking ability (Fig. 4A,B), even though complete haltere ablation decreases figure responses in open loop (Fig. 3A). However, the effects of haltere removal on closed-loop behavior are not as simple as an absence of response to wide-field motion. Flies with ablated halteres are better able to stabilize a wide-field pattern when a static or moving figure is present (Fig. 5A,B) and this result is not a function of the flies' figure tracking behavior alone, but rather of the interaction between the figure and the wide-field pattern (Fig. 5C,D). Further, the responses of haltereless flies to wide-field motion do show phase-locked oscillations (Fig. 3B): haltere removal does not effectively 'blind' the flies to wide-field motion, allowing them to focus their entire steering effort on figure



tracking. However, the decrease in wide-field response that follows haltere removal, combined with the fundamental trade-off between figure-tracking and the wide-field optomotor response (Fox et al., 2014), may cause the fly to shift its overall response toward figure-tracking behavior.

Wide-field optomotor responses are a well-characterized reflex in flies and many other animals. Figure-tracking behavior, in contrast, is generally considered to be a voluntary behavior. Voluntary, ‘outer-loop’ behaviors are often accompanied by efference copies, whereas ‘inner-loop’ reflexes are not, and this difference may explain why halteres are more influential over the reflexive behavior, which is unlikely to be affected by an efference copy. In whole-cell patch recordings of visual neurons during unidirectional wide-field stimulation, brief changes in the membrane potential coincide with fast wing-steering turns in the opposite direction of the visual stimulus (Schnell et al., 2014). These may be the manifestations of an efference copy, decreasing the responsiveness of the visual neurons during voluntary turns in the opposite direction of motion in order to dampen the reflexive optomotor behavior. This kind of counter-reflexive turning would be required to track a figure against a moving wide-field pattern. If haltere removal diminishes the optomotor reflex response to the wide-field stimulus more than it diminishes the voluntary tracking behavior, then the counter-directional rotation, as influenced by the efference copy, would increase. This could potentially result in stronger figure-tracking behavior in closed-loop tethered flight, but the fly’s decreased optomotor reflex would lead to instability in free flight. Further experiments, both behavioral and electrophysiological, will be necessary to determine how the reflex, voluntary and efferent motor signals are balanced, and how their dynamics interact to steer the fly. Our present data, however, further underlines the intrinsic separability of figure-tracking and optomotor behaviors (Reichardt et al., 1983; Egelhaaf, 1985; Lee and Nordström, 2012; Bahl et al., 2013; Fenk et al., 2014), and suggests differential multi-modal integration for their respective executions.

## MATERIALS AND METHODS

### Animals and technique

Female *Drosophila melanogaster* Meigen 1830, 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. 1A). In some flies, we removed the haltere end-bulb using a small pair of scissors (2 mm length cutting edge, Fine Science Tools, Foster City, CA), leaving the campaniform sensilla intact. In others, we removed the entire haltere including campaniform sensilla (Fig. 1B). Flies were allowed to recover for 30 min before experiments. Selected flies from each treatment group were filmed in flight with a high-speed video camera (Fastec Imaging, San Diego CA; supplemental material Movies 1–3). Flies were placed in the center of a cylindrical green LED flight arena, as described previously (Reiser and Dickinson, 2008). An infrared LED (Digikey, Thief River Falls, MN, USA) illuminated the wings, which cast a shadow on an optical sensor that converted the shadow into a voltage signal, and thus enabled the analysis of wingbeat amplitude and frequency (JFI Electronics, Chicago, IL, USA). The difference in amplitude between left and right wings ( $\Delta WBA$ ), as processed by this instrument, is proportional to the fly’s yaw torque (Tammero et al., 2004). Data were digitized at 1000 Hz (National Instruments data acquisition PCI card, Austin, TX, USA) and recorded using MATLAB (The MathWorks, Natick, MA, USA).

### Visual stimuli

We used two visual stimuli to test the effects of bulb or haltere removal on visually mediated wing-steering responses: a wide-field panorama in which the entire visual scene moved simultaneously, and a small moving figure (a 30-deg-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 presumably removing any salient figures from the wide-field pattern. The ‘on’ pixels were turned to the maximum luminance, creating high contrast between on and off LEDs. Both figure and panorama could be controlled in open-loop conditions by a prescribed function, or under closed-loop conditions by using the difference between the left and right wing beat amplitudes as a feedback signal. In closed-loop experiments, we presented the two stimuli independently: the fly controlled the motion of the figure, or controlled the motion of the wide-field pattern. Next, we presented two stimuli in which the fly controlled the wide-field motion independently of a figure that appeared in the frontal field: one in which the figure moved on an open-loop triangle-wave function, and one in which the figure was static. In closed-loop experiments, we varied the feedback gain such that steering produced higher or lower pattern rotation velocities. In experiments with multiple gains tested, each fly flew three trials at each gain. In control experiments (Fig. 4B,D; Fig. 5B,D and Fig. 6D–F), we measured the flies’ ability to stabilize their flight in the absence of wide-field visual input by repeating the above experiments with all of the LEDs in the arena turned on or off, or with a dark stripe moving against a homogeneously lit (all on or all off) background.

### Analysis

For open-loop experiments, we calculated the amplitude difference of left and right wingbeats as a metric of steering effort (Tammero et al., 2004). Kruskal–Wallis tests were used to assess differences between the groups. Stimuli (either figure or wide-field pattern) were moved by triangle-wave functions, and the peak-to-peak response magnitude was measured as a metric of response strength.

For closed-loop experiments, we measured the position of the figure or wide-field pattern as the fly steered it around the arena. The gain of the relationship between attempted steering and angular velocity was manipulated such that the pattern always moved opposite to the fly’s steering (as it would during free behavior), but did so with varying velocities relative to steering effort. At low gain, a given wing-steering effort resulted in slow pattern movement, and at high gain, the same effort produced faster movement.

When the fly moved a figure in closed loop, we computed mean vector strength, a circular statistic measuring angular dispersion (Batschelet, 1981; Reiser and Dickinson, 2010). Vector strength equal to one indicates that all flies positioned the figure at the same azimuth; lower vector strengths indicate a greater variance in the position of the figure. We used a Rayleigh *z*-test ( $P < 0.05$ ) to assess whether vector strength indicated that the distribution of azimuth angles were significantly different from random (Batschelet, 1981; Fox and Frye, 2014).

Wide-field motion is not a position-specific stimulus, so the flies are not expected to direct their flight towards a preferred location in the arena. Therefore, vector length is not useful in measuring stabilization ability when considering wide-field stimuli. If a fly is not stabilizing the pattern, the pattern will rotate around the arena. To estimate how well each fly stabilized the pattern, we calculated the number of times that the pattern made a complete rotation (a ‘spin’). We found the mean number of spins per trial for each treatment and used this number as an inverse metric of the fly’s ability to stabilize the pattern. Wilcoxon rank-sum tests were used to compute statistical differences between the groups for all closed-loop experimental conditions unless otherwise mentioned.

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### Competing interests

The authors declare no competing or financial interests.

### Author contributions

J.L.F. and S.M. designed experiments; S.M. performed research; J.L.F. and S.M. analyzed data; J.L.F. and S.M. wrote the manuscript.

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## Supplementary material

Supplementary material available online at  
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