

RESEARCH ARTICLE

Optomotor steering and flight control requires a specific sub-section of the compound eye in the hawkmoth, *Manduca sexta*

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ABSTRACT

While tracking odor plumes, male hawkmoths use optic flow cues to stabilize their flight movements with respect to their environment. We studied the responses of freely flying moths tracking odor plumes in a laboratory wind tunnel and tethered moths in an optomotor flight simulator to determine the locations on the compound eye on which critical optic flow cues are detected. In these behavioral experiments, we occluded specific regions of the compound eye and systematically examined the moths' behavior for specific deficits in optic flow processing. Freely flying moths with the dorsal half of the compound eye painted were unable to maintain stable flight and track the wind-borne odor plume. However, the plume tracking performance of moths with the ventral half of their compound eyes painted was the same as unpainted controls. In a matched set of experiments, we presented tethered moths with moving vertically oriented sinusoidal gratings and found that individuals with their eyes unpainted, ventrally painted and medially painted all responded by attempting optomotor-driven turns in the same proportion. In contrast, individuals with their compound eyes dorsally painted, laterally painted and completely painted showed no optomotor turning response. We decreased the contrast of the visual stimulus and found that this relationship was consistent down to a contrast level of 2.5%. We conclude that visual input from the dorso-lateral region of the moth's visual world is critical for successful maintenance of flight stability and that this species' visual environment must meet or exceed a contrast ratio of 2.5% to support visual flight control.

KEY WORDS: Vision, Tracking, Flight, Odor plume, Pheromone, Optic flow

INTRODUCTION

To successfully navigate through the world, animals depend on information about their movement with respect to their environment. Many animals detect this with their eyes by watching the wide-field motion cues generated as they move through their environment. This is particularly true for flying insects, which are suspended in a fluid medium with no fixed physical reference point from which to judge the direction in which they are moving. Flying insects have been shown to use visual information about their movement with respect to the fixed environment to control their flight speed and steering (Kennedy, 1940, 1951; David, 1979, 1982). Tasks that are known to be affected include responding to looming objects by either maneuvering to avoid obstacles or landing (Santer et al., 2005;

Tammero and Dickinson, 2002), and using the total amount of angular motion in the lateral visual field to judge distance traveled during foraging (Srinivasan et al., 2000).

Kennedy (1951) observed that migrating locusts in the field alter their groundspeed in response to altitude fluctuations, and proposed that they were controlling their speed over the ground by attempting to maintain a preferred velocity of visual motion across their eyes. In this mechanism, as the distance between the flying locust and the ground increases, the relationship between groundspeed and image velocity reaches a limit where the animal can no longer increase its flight speed to maintain the visual motion generated by the now distant ground, nor detect the movement of the ground owing to the resolution of their visual systems. Kennedy termed the altitude at which this occurs 'the maximum compensatory height' (Kennedy, 1951; Kuenen and Baker, 1982). Further research has shown an inverse relationship between the luminance and velocity of ventral visual motion necessary for tethered locusts to respond (Riley et al., 1988). Similarly, manipulating the movement velocity of the ground pattern below the fruit fly *Drosophila hydei* causes them to increase or decrease their flight speed (David, 1979). In a later experiment, David (1982) found that flies alter their flight speeds when the perceived velocity of their surroundings changes and when the perceived distance between the fly and the floor is altered suddenly. The flies altered their flight speed to maintain a constant angular velocity on the retina, confirming Kennedy's (1951) prediction. Rohrseitz and Fry (2011), using both modeling and behavioral data, were able to predict the observed change in *D. melanogaster* flight speed in response to visual perturbations in a novel free-flight closed-loop (when the presentation of the visual stimulus changes in response to the behavior of the animal) experiment. They found that flies would respond to changes in the wall patterns by altering their velocity, while increasing and decreasing the size of the elements in the floor pattern, simulating a change in altitude, which resulted in compensatory changes in flight altitude. In contrast, the findings of Straw et al. (2010) revealed that *D. melanogaster* used horizontal cues from nearby objects to stabilize their altitude, and responded to perturbations in these rather than to changes in optic flow. This shows that the mechanism that *D. melanogaster* uses may be tuned to different features than that of the locust, and that flies may use the visible elements of their environment differently in different contexts.

A study by Kuenen and Baker (1982) tested the idea of maximum compensatory height by challenging male oriental fruit moths, *Grapholita molesta*, to track pheromone plumes at different heights above the floor of a laboratory wind tunnel. As predicted, the moths increased their speed as their height above the floor increased, but then plateaued at a certain height. Kuenen and Baker (1982) suggested that once a certain height had been reached, these moths might have shifted their gaze from the pattern beneath them to other visual cues in their environment. Sanders et al. (1981) found that a forest insect, the spruce budworm moth, *Choristoneura fumiferana*,

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would not alter its flight speed in response to a moving visual pattern until the experimental pattern was shifted from the floor below the flying moths to above the transparent ceiling of their wind tunnel. Interestingly, the authors concluded that this prevented reflected light from washing out the visible pattern, not that the high contrast in the dorsal visual field was more important to this forest insect. Because insects cannot move their compound eyes in their heads, Kuenen and Baker's (1982) results may suggest that their moths selectively used wide-field motion inputs from only a subset of their compound eyes and were able to shift the part of the eye they used depending on the quality or reliability of the information detected. However, Moore et al. (1981) showed that honeybees, *Apis mellifera*, have a relatively narrow ventro-lateral zone of the compound eye, which is required for the detection of wide-field motion. If only this small subsection of the compound eyes was covered, the optomotor steering response of tethered honey bees was eliminated (Moore et al., 1981; Moore and Rankin, 1982). Conversely, if this narrow ventro-lateral section was left unpainted and the rest of the compound eye was covered, a normal optomotor steering response was observed (Moore et al., 1981; Moore and Rankin, 1982). This suggests that the brains of honeybees use primarily this portion of the visual world to maintain flight stability during navigation. Although experiments with freely flying bees have not been conducted, these results suggest that honeybees may not be able to shift their gaze associated with wide-field motion detection. Although they may adapt their flight maneuvers using wide-field motion from other parts of their eyes, this dorso-lateral region appears to be required, at least for the optomotor response.

It may seem surprising that something as important as wide-field motion detection is restricted to a dedicated zone of the compound eye, but there are well known examples of specialization of other functions of the compound eye in insects, which include: the polarized light-sensitive dorsal rim area (Homberg and Paech, 2002; Labhart, 1980; Labhart et al., 1984), the high-resolution 'fovea' of some predatory insects used for prey capture (Gonzalez-Bellido et al., 2011; Rossel, 1980; Rossel, 1983), and the enlarged dorsal ommatidia used by male flies to fixate on females for mating (Streinzer et al., 2013; van Praagh et al., 1980). In all of these examples, there are typically observably different sized ommatidia with different acceptance angles that distinguish these areas from the rest of the compound eye. In the case of the honeybee, the authors found nothing on the external surface of the optomotor-sensitive section of the compound eyes to obviously differentiate it from the rest of the eye (Moore et al., 1981).

One of the earliest experimental studies of pheromone plume tracking in male moths demonstrated that visual detection of wide-field motion was the basis for the moths' ability to orient into the wind and track the plume upwind (Kennedy and Marsh, 1974). Further studies have expanded and increased the resolution of this early work (Verspui and Gray, 2009; Vickers and Baker, 1994; Willis et al., 2011). One of these studies (Vickers and Baker, 1994) was aimed specifically at discovering what part of the visual world was essential for plume tracking. In that study, male *Heliothis virescens* moths tracked plumes of female pheromone to the source most successfully when they received visual stimulation from patterns above and to the sides at the 2 and 10 o'clock positions (with 12 o'clock directly overhead and 6 o'clock directly below). Unfortunately, in this experiment, the patterns were presented to the moths on the walls of an opaque cylinder, making it impossible to video record their flight behavior. Without being able to perform a detailed analysis of the moths' flight trajectories, it is extremely

difficult to understand exactly how these stimuli affected the animals' flight control.

Here, we have studied the behavioral responses of both freely flying and tethered flying moths to ask whether the reason moths track more effectively using a specific part of their visual world stems from functional specialization of their compound eyes. Our results show that both freely flying and tethered *Manduca sexta* (Linnaeus 1763) moths require the dorso-lateral visual field to respond to wide-field visual motion. Male moths that had the dorsal half of the compound eye occluded by acrylic paint were unable to successfully track a plume of female sex pheromone upwind to its source. In fact, most would not fly, and those that did landed quickly and never took flight again. The head turning and attempted steering of tethered flying moths in response to moving visual patterns was consistent with this behavior. Finer resolution of this experiment, covering just the medial or lateral portions of the compound eyes, revealed that by covering only the lateral portion of the eye, we significantly reduced attempted steering in response to wide-field motion stimuli. When all of the compound eye except the dorso-lateral intersection was painted, the proportion of moths responding to wide-field motion cues was not significantly different from the unpainted controls. From these results, we conclude that visual input from the dorso-lateral portion of the visual field is critical for flight steering responses supported by wide-field visual motion in *M. sexta* moths. These tethered flight studies also revealed that 2.5% is the lower limit for contrast detection in *M. sexta* under experimental conditions meant to simulate their flight activity period in nature.

MATERIALS AND METHODS

Moths and painting treatment

Prior to the beginning of normal scotophase, healthy 3- to 4-day-old male *M. sexta* moths were placed in clear plastic cups on crushed ice to anesthetize them while maintaining their light:dark cycle. When movement ceased, taking care to not damage the wings, animals were placed into a clamp to hold their wings still. Animals were placed into one of four groups ($N=25$ each): unpainted control, dorsally painted, ventrally painted and completely painted (Fig. 1A). For animals whose eyes were being painted, a coat of red enamel paint was applied first, followed by a layer of black acrylic paint. We used the red to make sure that we were completely covering the black compound eyes, and then covered the red with black paint to ensure complete obstruction of light to the affected areas (after Ye et al. 2003). For a schematic of how 'dorsal' and 'ventral' were determined, see Fig. S1. *Manduca sexta* ocelli are beneath the cuticle of the head (Eaton, 1971), so only unfocused light penetrating the scales and cuticle of the head could have been detected.

Free-flight experiments

After painting, the animals were placed in the dark at their normal lights off (LO) and experiments took place 2 h later, during their peak pheromone response period (Sasaki and Riddiford, 1984). The experimental arena was a 1×1×2.5 m laminar flow wind tunnel (Rutkowski et al., 2009; Willis et al., 2011; Fig. 1B) with a custom wooden turbulence mixing grid positioned at the upwind end of the working section (Talley, 2010). Animals were challenged to track a point source of one female equivalent of *M. sexta* sex pheromone gland extract on a 0.7 cm diameter disk of no. 1 filter paper (Whatman, Maidstone, UK) placed perpendicular to the wind direction. Animals were scored on their ability to perform the suite of behaviors associated with pheromone tracking [activation to

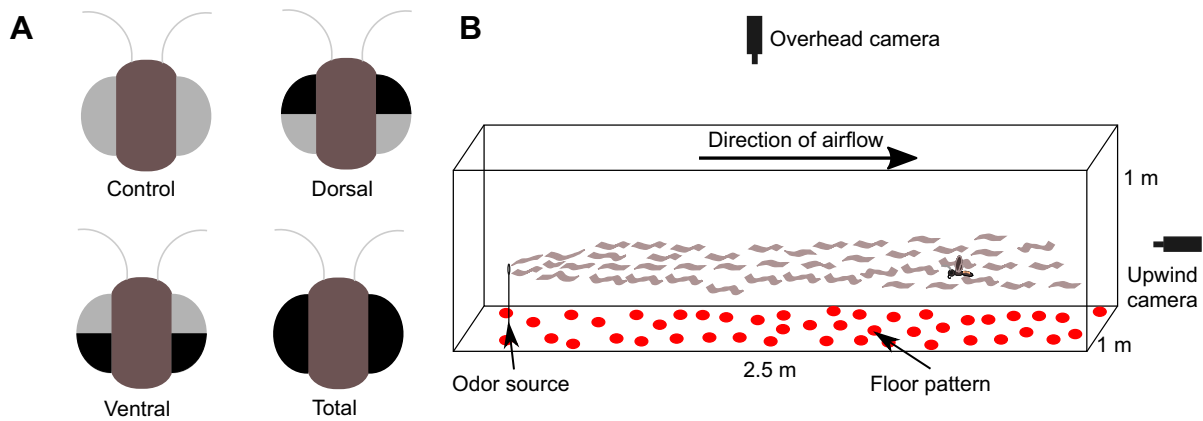


Fig. 1. The experimental eye-painting treatments and free-flight wind tunnel used in the experiments. (A) Frontal view of the experimental treatment groups ($N=25$ each) showing how the paint (shown here as black colored areas on the simplified face-on views) was applied to the compound eyes of the moths. For photographs of painting treatments, see Fig. S1. (B) A diagram of the wind tunnel used in the free-flight experiments. Video was recorded by an overhead camera placed above the wind tunnel and a second camera pointed toward the upwind direction.

pheromone, take off, locking on to the plume (i.e. lock on), tracking, track to source]. Flight behavior was recorded with two synchronized infrared-sensitive PointGrey (Richmond, BC) Firefly video cameras at the standard NTSC frame rate of 30 frames s^{-1} . We repeated these experiments in the absence of pheromone to analyze the flight behavior of experimentally manipulated moths in a non-goal-directed context.

Tethered experiments

After painting the compound eyes as described above (for treatments, see Fig. 2A), the ventral thoraces were descaled between the bases of the mid- and hind legs. Animals were then ventrally tethered to a rod using super glue and bonding accelerator, and placed in the dark for 30 min to allow their compound eyes to dark adapt. The animals were placed in a flight simulator, similar to the setup described in Gray et al. (2002) (Fig. 2B). In our setup, one computer was connected to a projector that projected the stimulus

(generated by the PsychToolbox MATLAB package) onto the outside of a plastic dome 66 cm in diameter. The tethered moths were positioned inside the dome facing the projected patterns and presented with moving vertical sinusoidal patterns at a luminance of 1 cd m^{-2} that varied by temporal and spatial frequency, and contrast. Contrast was defined using the Michelson contrast, which can be defined as follows:

$$\frac{I_{\max} - I_{\min}}{I_{\max} + I_{\min}}, \quad (1)$$

where I_{\max} is the highest luminance and I_{\min} is the lowest luminance. Previous experiments (Parthasarathy and Willis, 2018) show that this luminance results in peak optomotor response in dark-adapted tethered flying *M. sexta*. The pattern moved rightward for 3 s, then switched to a leftward motion for the next 3 s, giving a total trial length of 6 s. The trial periods were recorded via a

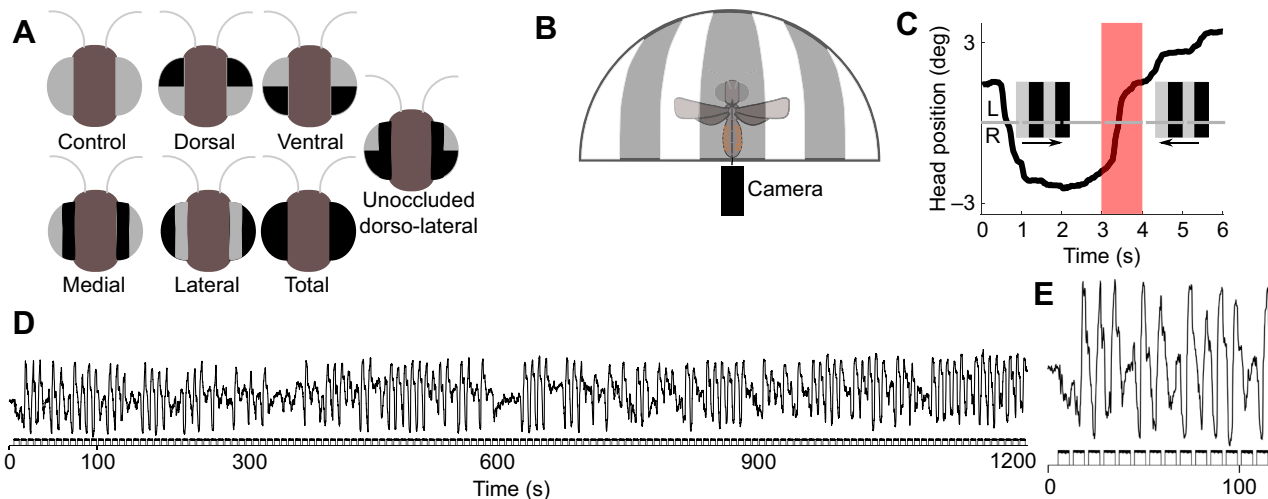


Fig. 2. The tethered flight experimental setup shown with the treatment groups and raw behavioral responses. (A) Experimental groups ($N=10$ each) used in the tethered optomotor experiments. In addition to the four groups shown in Fig. 1, there was the addition of insects with either their medial (towards the head) or lateral (away from the head) compound eyes painted. Another group, which had the entire compound eye painted, with the exception of the dorso-lateral intersection, was also introduced in this set of experiments. (B) Diagram of an overhead view of the flight simulator. (C) Trace of an individual 6-s trial. Red shading indicates the time period in which the success/fail criteria (80% maximum) was judged. Two schematics show the direction of movement in each 3-s half. (D) Sample trace of an entire experiment of one control moth. Top trace indicates head movement. Bottom trace indicates individual 6-s trials with 2-s breaks. (E) A magnified view of the first 100 s of D.

photodiode that sent a signal to a Neuralynx data acquisition system (Neuralynx, Bozeman, MT, USA).

Data analysis

In the free-flight wind tunnel experiments, moth flight trajectories were digitized and three-dimensional reconstructions of the trajectories constructed using the DLTdv5 MATLAB script (Hedrick, 2008). We then measured a set of parameters typically used to quantify odor plume tracking flight trajectories (Marsh et al., 1978; Rutkowski et al., 2009). During the tethered flight arena experiments, the head turning movements associated with attempts to turn were recorded on video and digitized with a custom MATLAB script (Fig. 2C). A ‘response’ was defined by the presence of a change in head position that was >80% of the maximum over the 3–4 s period (Fig. 2D,E).

We used Fisher’s exact test to compare proportions of individuals successfully reaching the pheromone source (using Bonferroni corrections for cases with more than two groups). To compare computed flight kinematics and the responses of tethered behavior, we used pairwise Wilcoxon–Mann–Whitney (WMW) tests with Bonferroni corrections. Statistical tests were performed with R (<https://www.r-project.org/>).

RESULTS

Dorsal visual input is required for free-flight odor tracking

We first experimentally manipulated three groups of healthy age-matched male *M. sexta* moths to restrict their detection of different parts of their visual world. To achieve this, we covered the dorsal half of the compound eyes (i.e. dorsally painted), the ventral half of the compound eyes (i.e. ventrally painted) and the entire compound eye area (i.e. total occlusion) (Fig. 1B). An age-matched group of moths whose eyes had not been painted comprised a fourth ‘control’ group. These four experimental groups were each challenged to track a plume of female sex pheromone upwind in our laboratory wind tunnel (Fig. 1A). Unpainted controls and moths that had ventrally painted eyes successfully took flight and tracked pheromone plumes with similar success rates (Fig. 3A). In contrast, only 1 (4%) of the moths with dorsally painted eyes was able to successfully track the odor to its source (Fig. 3A). The reported proportion of successes is significantly lower than the proportion of control animals that successfully completed this task (73.1%, Fisher’s exact test, $P < 0.05$) and comparable to that of the moths with completely painted compound eyes (i.e. 0%) (Fig. 3A).

This animal, however, did not perform the full suite of plume track behaviors and landed after only a brief period of tracking. Because it did not track the plume continuously, and only regained flight just below the source and began to hover, it was excluded from future analysis.

To control for the possibility that the paint we used might have affected the moths beyond occluding the specified parts of their visual inputs, we painted the entire compound eyes of a group of moths with clear acrylic medium. This is the acrylic paint we used with no pigment in the formulation. There was no statistically significant difference in the proportion of moths in this group that were able to track the plume to the source compared with unpainted controls (87% intact controls versus 81% clear acrylic medium, Fisher’s exact test, $P > 0.05$; Fig. 3B). This indicates that the decreased performance we observed in our eye painting treatments is caused by eliminating visible inputs from the parts of the compounds eye we covered with paint.

It is probable that tracking an odor plume is a specific task requiring specific sensory inputs. To test for this possibility, we challenged the moths to fly in the wind tunnel in the absence of a pheromone plume, and measured their ability to take off and successfully maintain controlled flight in clean air. For the purposes of this experiment, controlled flight was defined as the condition in which the moth maintained a consistent altitude without falling to the floor of the arena, which would occur when take-off was successful. As in our plume tracking experiments, the controls and ventrally painted moths flew similarly and very well (93% of controls and 87% of ventrally painted moths took off and flew in a controlled way, Fisher’s exact test, $P > 0.05$; Fig. 3C). A significantly lower percentage of dorsally painted and completely painted moths (6.25% for each, Fisher’s exact test, $P < 0.05$) was able to achieve controlled flight. Overall, the moths in each of our treatment groups showed similar levels of flight control whether plume tracking or flying in clean air.

Moths able to track the plume exhibited similar behavior

During experiments, we used video cameras to record the flight tracks and subsequently quantified the trajectories of individuals successfully tracking the plume to its source. Our analysis showed that there are no statistically significant differences between control and ventrally painted individuals in any of the measured flight track parameters (pitch, yaw, course, track and drift angles, airspeed and groundspeed, WMW, $P > 0.05$), showing that, according to the

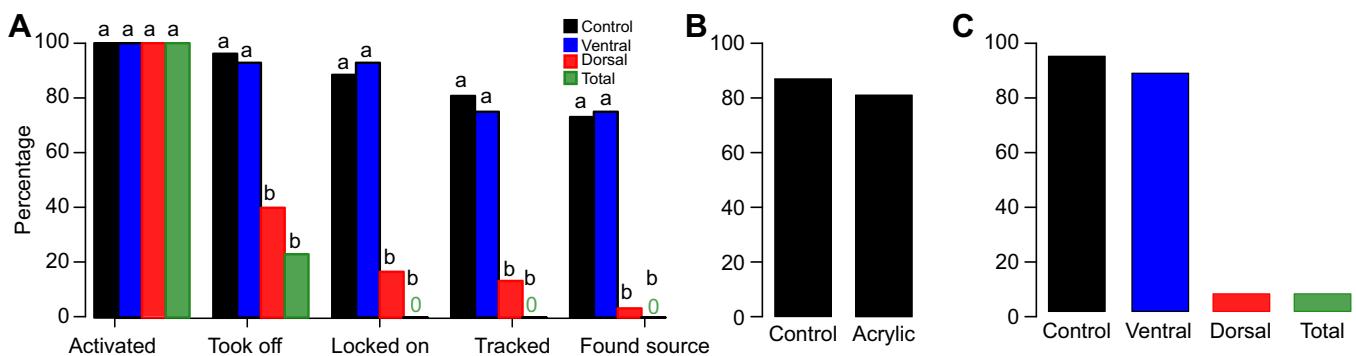


Fig. 3. Percentage of experimental population performing each behavior in the sequence from pre-flight activation to locating the pheromone source in wind+odor experiments, and those exhibiting stable controlled free flight in no wind and no odor. (A) Percentages of the four groups over the sequence of behaviors in plume tracking. Significance letters denote comparisons with controls using pairwise Fisher’s exact tests with Bonferroni corrections. (B) Percentage of successful tracks between controls and individuals with full clear acrylic eye covering. (C) Percentage of individuals successfully maintaining controlled flight in clean air.

parameters we measured, lack of visual input from the ventral compound eyes does not affect the plume tracking flight of *M. sexta* males (Fig. 4). Because only one moth with the dorsal parts of their compound eyes covered, and no moths with the entire surface of the eye covered, tracked the plume, it was impossible to compare them with the other treatments in this way.

Dorsal and lateral visual input is necessary for successful optomotor behavior

To more precisely control the moths' exposure to wide-field visual motion, we tethered them and placed them in a flight simulator used previously for *M. sexta* flight studies (Gray et al., 2002) (Fig. 2B). The tethered flying moths were then presented with wide-field

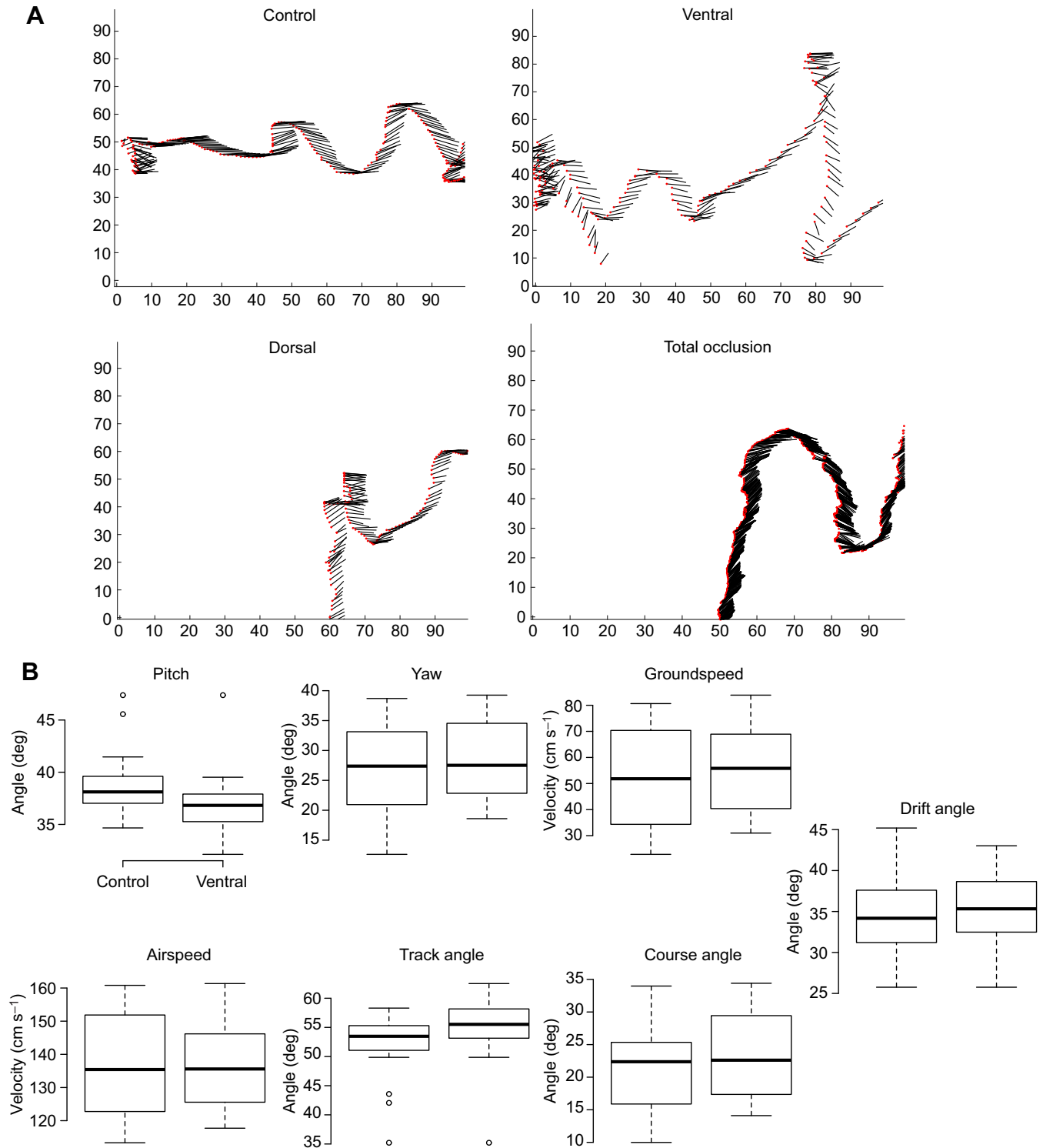


Fig. 4. Comparison of flight tracks and median flight kinematics. (A) Sample flight tracks viewed from overhead. Red dots denote head and black lines denote angle of the longitudinal body axis. (B) Box plots measured from control (left) and ventrally painted (right) moths. Circles denote outliers. No statistical differences between groups were found using Wilcoxon–Mann–Whitney tests ($P > 0.05$).

visual motion of varying temporal and spatial frequencies and contrasts at a light level approximating sunset (1 cd m^{-2}). This is the time at which they typically fly in nature and the light level at which *M. sexta* have peak contrast sensitivity (Stöckl et al., 2017; Parthasarathy and Willis, 2018). In addition to the previous four groups tested in the free-flight experiments, we tested animals with either the lateral or medial portions of both compound eyes painted (Fig. 2A). We measured the proportion of successful head turning responses and found that from 2.5 to 20% contrast, there are no significant differences between control, ventrally painted and medially painted individuals (Fig. 5). In contrast, completely painted, dorsally painted and laterally painted individuals showed significantly lower response rates. Finally, we painted the entire compound eye with the exception of the dorso-lateral section (see diagram in Fig. 3) and found that there was no significant difference in attempts to turn in response to wide-field motion stimuli between these individuals and unpainted controls (Fig. 4).

Optomotor performance is unaffected down to 2.5% contrast

As can be seen in Fig. 5, the proportion of the three groups that successfully responded to the moving patterns (control, ventral and medial) are not significantly different from one another for contrasts from 20 to 2.5%; however, once the contrast is lowered to 1%, they perform at the same proportion as the three unsuccessful groups (dorsal, lateral and total, pairwise WMW tests with Bonferroni corrections, $P > 0.05$). This indicates that the lower limit of contrast sensitivity for wide-field visual detection for this species at 1 cd m^{-2} light level is 1%.

DISCUSSION

A lack of dorso-lateral visual input eliminates optomotor behavior

We found that in both free-flight and tethered optomotor experiments, dorsally painted individuals behaved as if we had removed all visual input by completely covering their compound eyes (Figs 3A and 5). Animals with the lateral portions of the

compound eye painted showed the same lack of behavioral response in the tethered flight experiments (Fig. 4). When animals had no visual input except the dorso-lateral compound eye, they continued to perform visually driven steering responses as if their eyes were free of paint. Vickers and Baker (1994) found that male *H. virescens* moths located a source of female pheromone at the highest proportion when visual patterns (i.e. dark spots on a light background) were presented above them (at the 2 and 10 o'clock positions of the cylinder that the moths flew through). Visual patterns at this position in the environment certainly could be detected by the dorso-lateral compound eye.

Compared with the honeybee, which requires ventro-lateral visual input for successful optomotor behavior (Moore and Rankin, 1982), it is somewhat surprising at first that the dorsal area of the compound eye is utilized by *M. sexta*. In the shield bug *Parastrachia japonensis*, a nocturnal insect, the dorsal visual cues from the canopy are used in navigation to and from their burrow. It is possible that nocturnal insects use the dorsal view from their compound eyes because that view will provide maximum contrast between the skylight and any other part of the environment. Emphasizing the overhead view could also compensate for the limited availability of light at night. A comparative study on a diurnal species related to *M. sexta* would be informative to test this hypothesis.

Another possible explanation of the observed dorsal-ventral asymmetry could be that nocturnal hawkmoths have an asymmetry in visual acuity between the dorsal and ventral regions of the compound eye. If the ventral half of the animal's visual world receives less input than the dorsal half, owing to lack of contrast or altitude of flight above the ground, then it may have developed to have a lower visual acuity. However, the ventral half of the compound eye has a higher concentration of blue photoreceptors, compared with the dorsal half (Bennett et al., 1997; White et al., 2003), and *M. sexta* has an innate preference to feed from flowers with a blue reflectance (Cutler et al., 1995; Goyret et al., 2008). This has led to the hypothesis that an area in the ventral compound eye is

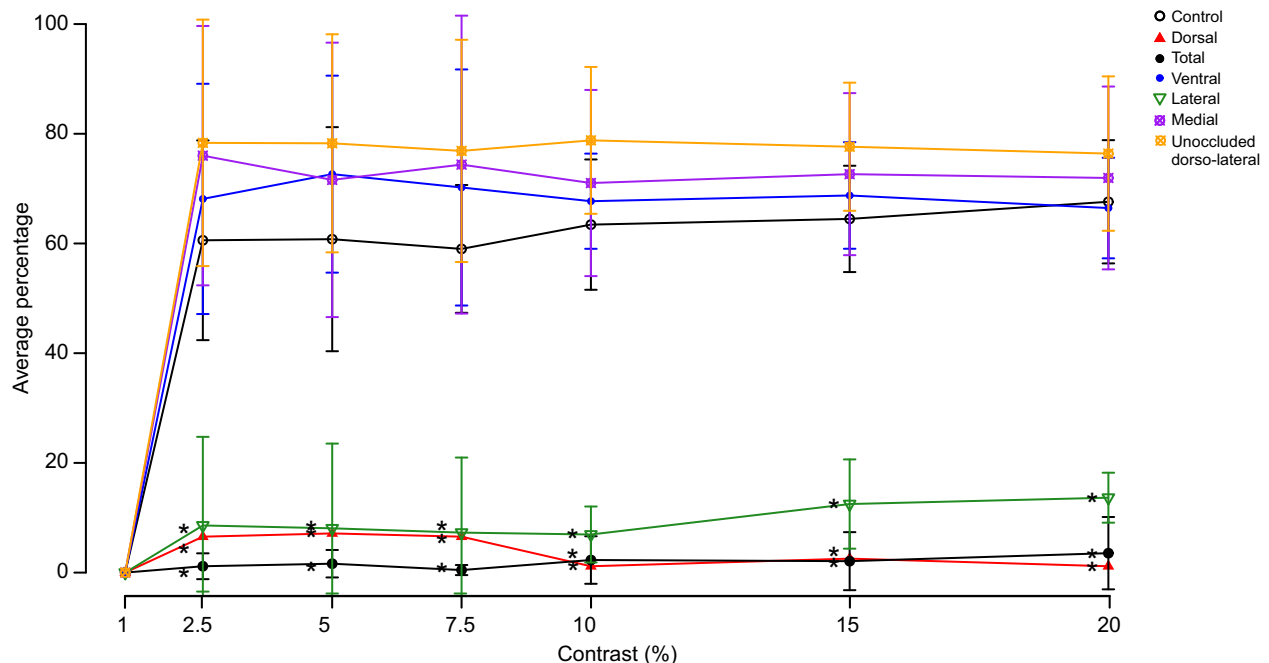


Fig. 5. Mean (\pm s.d.) response rates of each experimental treatment group (see details in Fig. 2) measured at each contrast level. Asterisks denote column-wise significant differences (pairwise Wilcoxon–Mann–Whitney tests with Bonferroni corrections $P < 0.05$) from control groups at that contrast level.

specialized for feeding (Bennett et al., 1997; Cutler et al., 1995). Both sexes of *M. sexta* actively forage for nectar and feed from night-blooming flowers while flying, and this requires precise visually guided maneuvers (Raguso et al.; Sponberg et al.).

Plume tracking and optomotor behaviors unaffected by lack of ventral input

Our results reveal that in free-flight experiments, ventrally painted individuals performed as well as unpainted controls in both flight maneuvering (Fig. 4) and success in locating the source (Fig. 3A). This relationship between ventrally painted individuals and unpainted controls was substantiated by the tethered flight experiments. These results are surprising when compared with previous studies showing that manipulation of a ground pattern can alter the groundspeed and steering of moths (Kennedy and Marsh, 1974) and flies (David, 1979).

Unlike the explanation proposed in Kuenen and Baker (1982), where once the moths reached a particular height above the floor pattern their increase in flight speed plateaued, suggesting that input from other regions of the environment was then used, there does not appear to be any flexibility prioritizing the visual inputs to flight control in *M. sexta*. This rigidity in the response to the location of wide-field motion cues is similar to the results in studies of the honeybee (Moore et al., 1981; Moore and Rankin, 1982). However, other experiments in our laboratory have shown that changes in the ventral visual world do cause *M. sexta* to alter their flight behavior (Willis and Arbas, 1995). It may be that, although visual input from the dorso-ventral compound eye is required, if other visual information is available, it is utilized. It would be informative to compare the effects of visual perturbations in the ventral and dorsal visual world, while taking care to prevent reflection from the walls and ceiling of our wind tunnel. These experiments are ongoing.

Alternatively, this apparent contradiction in the case of the wind tunnel experiments could be explained by the pattern from the floor of the arena being reflected onto the Plexiglas ceiling in our experimental conditions, similar to the situation proposed by Sanders et al. (1981). It is also typical for free flight wind tunnel experiments to control the experimentally manipulated visual component of the environment (i.e. the floor) while the other transparent parts of the wind tunnel (i.e. walls and ceiling) remain unaltered. This means that other objects in the wind tunnel room may be visible to the moths through the transparent walls and ceiling of the wind tunnel, making it possible for them to use other parts of their visual world for flight control.

In light of our results, another question that arises is the function of the ventral compound eye. Mazo and Theobald (2014) found that tethered *D. melanogaster* responded strongly to translational optic flow presented to the lower half of the visual field and the entire visual field, but less so when presented to the upper half. Their conclusions confirmed a prediction by Krapp and Hengstenberg (1996) that rotational and translational optic flow detection may be different information streams. This hypothesis is supported by the discovery of a neural correlate in that visual interneuron VT1 fires spike bursts when presented with translational cues (Longden et al., 2017). It would be interesting to perform a set of experiments similar to those of Mazo and Theobald (2014) to see whether this model holds true for moths. It would also be interesting for us to perform plume tracking experiments on ventrally painted moths with decreasing altitudes above the floor of the odor plume to see whether we can observe the effects of a loss of translational motion in *M. sexta*. One potential caveat with this experiment, however, would be that moving the odor plume too close to the floor could

alter the plume structure, complicating comparisons between experiments.

Another potential explanation is that the dorsal facets are upstream of the ventral area, such that removing dorsal input also causes defects in the ventral area. Future electrophysiological experiments could elucidate this by testing whether removing dorsal visual input causes lobula plate tangential cells with receptive fields in the ventral half to be inhibited.

There is an indication that something like this might be happening in day-flying hover flies, *Episyrphus balteatus*. A recent study shows that altering the position of the dominant light source affects the ability of hover flies to stabilize their flight (Goulard et al., 2018). In this case, the authors suggest that input from the dorsal part of the eyes is the sensory information needed for the animal to orient dorsal side up and ventral side down. Without this basic sensory cue, the hover fly's ability to stabilize using visible wide-field motion cues is impaired. However, the reported impairment measured is relatively subtle compared with the essentially complete disruption in the ability to fly without dorsal input in *M. sexta* males.

Contrast sensitivity is maintained down to 2.5%

We found that the limit of contrast sensitivity at a luminance of 1 cd m^{-2} is 2.5%. At a contrast below this (1%), there was no detectable response to wide-field visual motion. This is the lower limit in contrast sensitivity in *M. sexta* at the light level at which they are normally active in the field. In other behavioral experiments, *M. sexta* consistently did not respond to stimuli with a contrast of 1% at all measured light levels, from daylight to starlight (Parthasarathy and Willis, 2018). Previous recordings from the lobula plate tangential neurons in the brain of *M. sexta* (Stöckl et al., 2017) showed that reliable responses stopped at 5% contrast at the same light levels we used (1 cd m^{-2}). This difference may be explained by differences in the experimental apparatus used to deliver the visual stimuli. Though the moths used in Stöckl et al. (2017) and the present study originated from the same colony, the stimulus apparatus used may have important differences. Stöckl et al.'s (2017) wide-field motion stimuli were presented to preparations where behavior was not possible, and the stimuli themselves were presented to primarily the frontal field of the compound eyes using a flat panel monitor. In our experiments, the tethered moths were stimulated by a hemispherical back-projection screen that wrapped around them, stimulating much of the compound eye. Future electrophysiology experiments could study the responses of wide-field motion-sensitive neurons with receptive fields in the dorso-lateral compound eye to determine whether these neurons have a lower contrast sensitivity.

Compared with recordings in hoverfly HS cells in a luminance more than an order of magnitude brighter (41 cd m^{-2}), where the authors found no responses in contrasts below 0.129, *M. sexta* is more sensitive to contrast (Straw et al., 2006). Given that the conditions in which *M. sexta* operates are significantly darker, it would make sense that our moths would have adapted to detecting lower contrasts. Stöckl et al. (2017) also showed that the diurnal hawkmoth *Macroglossum stellarum* had significantly lower contrast sensitivity at luminances below 10 cd m^{-2} , with no discernable responses below 0.01 cd m^{-2} .

Conclusions

We have determined that the dorsal half of the *M. sexta* compound eye is required for free-flight odor tracking (Fig. 3). We have also used a tethered flight paradigm to determine that a small subsection of dorso-lateral ommatidia are necessary to successfully respond to

wide-field motion stimuli (Fig. 5). Finally, we have also shown that *M. sexta* is able to respond to wide-field motion with contrasts as low as 2.5% at levels of illumination characteristic of their normal active period in nature (Fig. 5).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.C., M.A.W.; Methodology: S.C., K.P., M.A.W.; Software: S.C., K.P.; Validation: S.C.; Formal analysis: S.C.; Investigation: S.C., M.A.W.; Resources: M.A.W.; Data curation: S.C.; Writing - original draft: S.C., M.A.W.; Writing - review & editing: S.C., K.P., M.A.W.; Funding acquisition: M.A.W.

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Supplementary information

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