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Visual feedback influences antennal positioning in flying hawk moths

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ABSTRACT

Insect antennae serve a variety of sensory functions including tactile sensing, olfaction and flight control. For all of these functions, the precise positioning of the antenna is essential to ensure the proper acquisition of sensory feedback. Although antennal movements in diverse insects may be elicited or influenced by multimodal sensory stimuli, the relative effects of these cues and their integration in the context of antennal positioning responses are not well understood. In previous studies, we have shown that fields of Böhm's bristles located at the base of the antennae provide crucial mechanosensory input for antennal positioning in flying hawk moths. Here, we present electrophysiological and behavioral evidence to show that, in addition to the Böhm's bristles, antennal muscles of hawk moths also respond to bilateral visual input. Moreover, in contrast to the mechanosensory-motor circuit, which is entirely contained within the ipsilateral side, visual feedback influences antennal positioning on both contralateral and ipsilateral sides. Electromyograms recorded from antennal muscles show that the latency of muscle responses to visual stimulation ranged from 35 to 60 ms, considerably slower than their responses to mechanosensory stimuli (<10 ms). Additionally, the visual inputs received by antennal muscles are both motion-sensitive and direction-selective. We characterized the influence of visual feedback on antennal positioning by presenting open-loop translational and rotational visual stimuli to tethered flying moths. During rotational stimuli, we observed that the antenna contralateral to the direction of the turn moved forward through larger angles than the ipsilateral antenna. These observations suggest that whereas input from the Böhm's bristles mediates rapid corrections of antennal position, visual feedback may be involved in slower, bilaterally coordinated movements of the antenna during visually guided flight maneuvers. Thus, visual feedback can modulate the set point at which the antenna is held during flight in hawk moths.

KEY WORDS: Antennal positioning behavior, Böhm's bristles, Vision, Antennal mechanosensors

INTRODUCTION

The onset of flight in diverse insects is accompanied by a forward positioning of the antennae. In moths, this behavior involves moving the antennae forward from rest to a flight position and maintaining this position during flight bouts (Dorsett, 1962). Antennal positioning is primarily mediated by the Böhm's bristles at the base of the antenna, which provide mechanosensory feedback to the antennal muscles at time scales of less than half a wing stroke (Krishnan et al., 2012). A complementary set of antennal

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mechanosensors, the Johnston's organs, sense antennal vibrations and are involved in flight control (Sane et al., 2007). Because antennal positioning mediated by the Böhm's bristles may enable Johnston's organs to unambiguously sense the antennal vibrations, it likely plays a crucial, albeit indirect, role in flight control.

In addition to providing inputs necessary for flight control [e.g. odonates (Gewecke et al., 1974), lepidopterans (Sane et al., 2007; Sane et al., 2010), dipterans (Mamiya et al., 2011)], insect antennae are also involved in olfaction, tactile sensation, gravity and wind sensing, thermosensation and hygrosensation (Comer et al., 2003; Gewecke and Niehaus, 1981; Kamikouchi et al., 2009; Niehaus, 1981; Schneider, 1964; Ye et al., 2003). Proprioceptive feedback about antennal position may be crucial for many of these sensory functions. In cockroaches, scapal hair plates similar to the Böhm's bristles encode the position of the antenna during active tactile sensing (Okada and Toh, 2000; Okada and Toh, 2001; Okada and Toh, 2006). Moreover, many antennal movements occur in concert with visual feedback, which is also important in flight behavior (e.g. Verspui and Gray, 2009). For instance, the antennae of many insects may be readily observed to track moving objects close to them [e.g. cockroaches (Ye et al., 2003), crickets (Honegger, 1981), bees (Erber et al., 1993)]. These observations suggest that the antennal motor system is likely influenced by visual cues in addition to mechanosensory cues.

In this paper, we used a combination of electrophysiological and behavioral techniques to show that visual cues influence antennal positioning in the Oleander hawk moth, *Daphnis nerii* (Linnaeus 1758). Electromyogram recordings from antennal muscles revealed that the antennal muscles respond in a motion-sensitive and direction-selective manner to visual cues. In addition, behavioral experiments showed that tethered flying moths modulate their antennal position in response to moving visual stimuli. Collectively, these data suggest that antennal positioning is a multimodal reflex in which the Böhm's bristles serve to maintain antennal position on stroke-to-stroke time scales, whereas the visual feedback modulates and bilaterally coordinates the antennal positions during visually guided flight maneuvers at slower time scales.

RESULTS

Antennal muscles receive bilateral visual feedback

We recorded electromyograms from the Ms-pp intrinsic muscle (Niehaus and Gewecke, 1978) of the left antenna while providing 10 ms pulses of white light from an LED first to the ipsilateral eye and then to the contralateral eye (Fig. 1A–I; N=10). The raw electromyograms were band-pass filtered and spike-sorted (supplementary material Fig. S1). The muscle showed excitatory responses (Fig. 1B,C) to both ipsilateral (Fig. 1D) and contralateral (Fig. 1E) visual stimulation, in contrast to the responses to stimulation of the Böhm's bristles, which were ipsilaterally confined (Krishnan et al., 2012). The excitatory response typically consisted of an initial burst of spikes following the stimulus, followed by return to the baseline levels (Fig. 1F–I). The representative case (Fig. 1B,C) showed
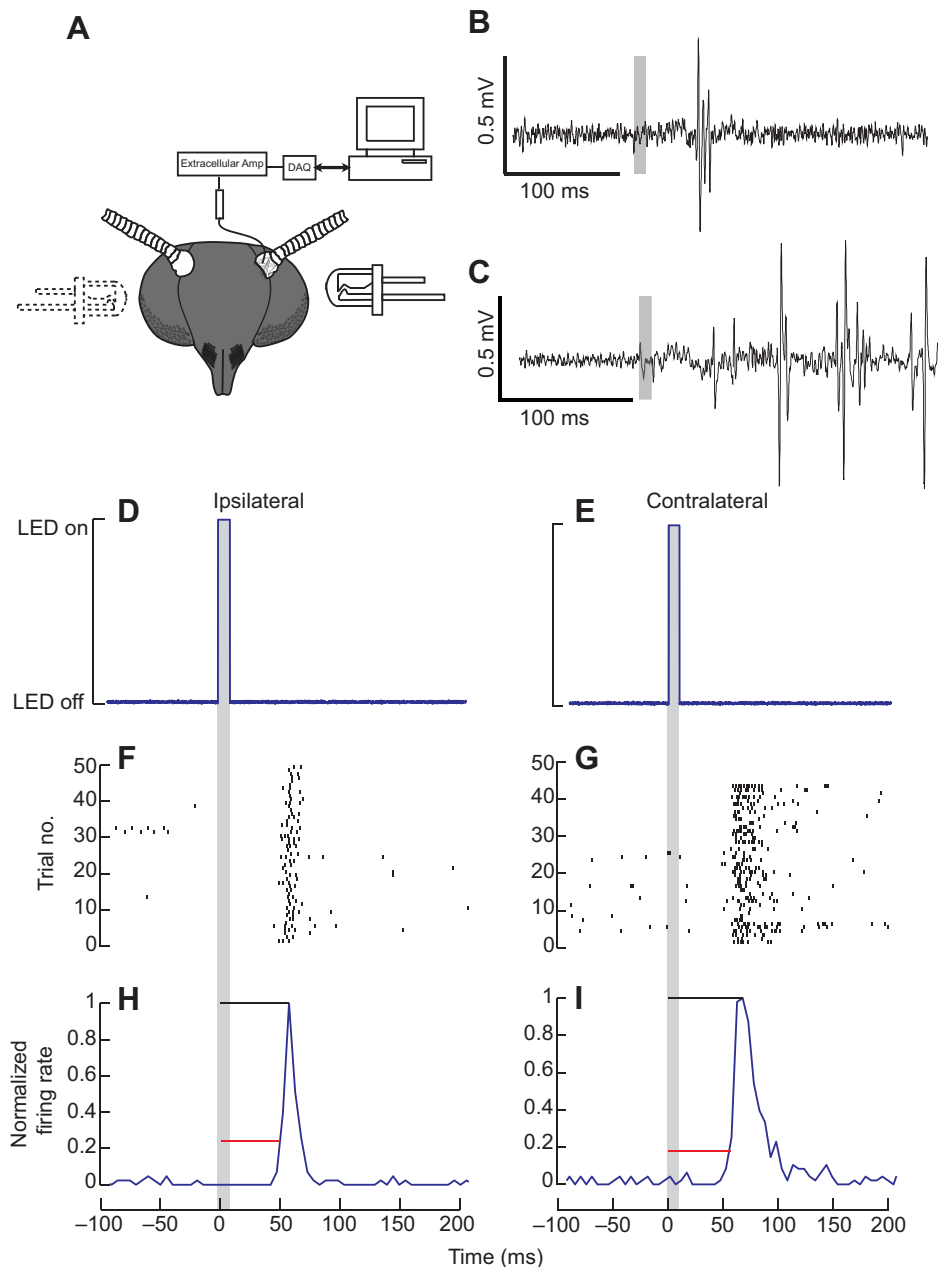
Fig. 1. Bilateral visual input to the antennal muscles. (A) Schematic diagram of electromyogram (EMG) recordings from the Ms-pp intrinsic antennal muscle while stimulating the ipsilateral eye (solid LED) and the contralateral eye (dashed LED). (B,C) An example of spiking responses in intrinsic antennal muscles after stimulation of the ipsilateral (B) and the contralateral eye (C). The traces shown are band-pass filtered (see Materials and methods). The overall structure of these responses was variable (see supplementary material Fig. S2). (D,E) A 10 ms white light LED pulse (gray bar, beginning at 0 on the time axis) was used as a visual stimulus. (F–I) Representative spiking raster plots (F,G) and normalized peri-stimulus time histograms (H,I). Although response latencies were calculated using 1 ms bins, the histograms in this figure were generated with 5 ms bins for visual purposes. Shown here are the responses of intrinsic muscles to stimulation of the ipsilateral eye (F,H) and contralateral eye (G,I). The red lines on the histogram represent the latency to significant firing shift, and black lines the latency to peak firing rate.

a much stronger response to contralateral visual stimulation, with larger-amplitude spikes firing much later than to ipsilateral visual stimulation. The structure of the response could be quite variable between preparations, and included cases in which the muscle responded earlier to contralateral stimulation, or in which the response to ipsilateral visual stimulation was stronger (supplementary material Fig. S2).

Visual input to the antennal muscles is slower than mechanosensory input

After binning the data into 1 ms time bins and normalizing them to their peak value, we calculated the response latency of antennal muscles to visual stimuli. We calculated the 'latency to significant firing shift', or the time difference between the stimulus start point and the point where the firing rate crossed 5 s.d. from the mean, and the 'latency to peak firing rate', which was the time taken for the normalized firing rate to reach its peak value of 1.

In response to ipsilateral visual stimulation, the Ms-pp muscle significantly increased its firing rate in 60.7 ± 31.2 ms (median 53.0 ms), while the peak firing rate was reached in 68.7 ± 29.5 ms (median 61.3 ms, $N=10$; Fig. 2A,B). Contralateral visual stimulation resulted in a significant shift in firing in 45.6 ± 11.7 ms (median 44.2 ms), and peak firing was achieved in 68.0 ± 9.8 ms (median 66.0 ms, $N=10$; Fig. 2A,B). Latencies to firing shift were significantly higher ($P<0.01$; Fig. 2C) than those reported for intrinsic muscle responses to stimulation of the Böhm's bristles (Krishnan et al., 2012). Additionally, the response latencies to ipsilateral and contralateral visual stimulation were not significantly different from each other.

Antennal muscles receive information about the direction of visual field motion

Our next goal was to understand the nature of the information conveyed by the eyes to the antennae. A neuroanatomical study in
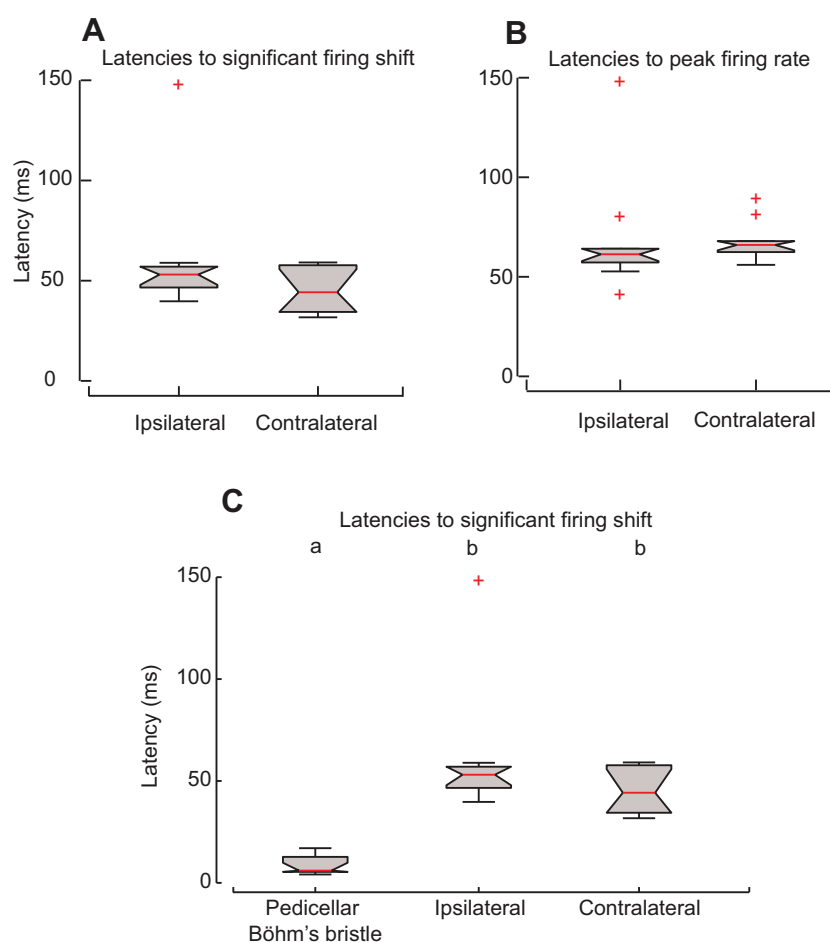
Fig. 2. Response latencies of antennal muscles to visual stimuli. (A,B) Notch plots showing the distribution of latencies to significant firing shift (mean + 5 s.d.) (A) and to peak firing rate (B) of the Ms-pp intrinsic muscle in response to ipsilateral or contralateral visual stimulation (*N*=10), with statistical outliers represented as red plus signs. The median line represents the median latency (see Results), while the whiskers represent the highest and lowest latencies recorded during our experiments (minus outliers). Response latencies were similar irrespective of which eye was stimulated. (C) Responses of intrinsic muscles to visual stimuli (ipsilateral/contralateral) occurred at significantly longer latencies (b, *P*<0.01) than responses to mechanosensory stimuli delivered to the Böhm's bristles (a) [from previously published data (Krishnan et al., 2012)].

honeybees showed an overlap between lobular interneurons and antennal sensory afferents (Maronde, 1991). To understand the role of wide-field visual input in antennal positioning of hawk moths, we used bilaterally placed 2D gratings displayed on monitors to provide four types of open-loop visual stimuli to an immobilized moth. The motion stimuli were: forward translation (backward movement of the visual field), backward translation (forward movement of the visual field; Fig. 3A,B), clockwise rotation (counterclockwise movement of the visual field) and counterclockwise rotation (clockwise movement of the visual field; Fig. 4A,B). We simultaneously recorded electromyograms (EMGs) from antennal muscles to record their response to each type of motion (*N*=13). Unilateral motion stimuli failed to evoke a muscle response, regardless of the direction of stimulation or which eye was stimulated (supplementary material Fig. S3).

In most cases, the intrinsic muscles showed excitatory responses to motion stimuli, often with a clear preferred direction of motion. When motion of the visual field was opposite to the preferred direction of motion, responses were either weakly excitatory or weakly inhibitory (see Fig. 3A,B for a representative case that responded preferentially to backward translation). However, in some cases, the responses did not have a significant directional bias (Fig. 4A,B; see supplementary material Fig. S4 for additional examples). We quantified the change in firing rate for each moth in response to each of the four stimuli. Δbwd represents the average change in firing rate in response to backward translation, Δfwd to forward translation, Δccw to counterclockwise rotation and Δcw to clockwise rotation.

We compared the values of Δfwd versus Δbwd, and Δccw versus Δcw to determine the degree of directional bias in the response to motion. Out of 13 different recordings of responses to translation, 11 showed a response that was significantly biased toward backward translation (Fig. 3C, red), whereas one more was similarly biased but not significantly so (Fig. 3, black). From all recordings, we obtained a response biased toward forward translation just once (Fig. 3C, blue). The responses to open-loop rotations were more evenly distributed, with four out of 13 responses showing a preference for counterclockwise rotation (Fig. 4C, blue), and another four biased toward clockwise rotation (Fig. 4C, red). In five cases, we observed an equal response to the two directions of rotation, with no significant bias (Fig. 4C, black). The variation in directional selectivity within the same muscle suggests that each antennal muscle receives input from multiple directionally selective motion detectors.

Motion of the visual field modulates antennal position during flight

The presence of motion-sensitive input to the antennal muscles raises the possibility that visual input is important in either maintenance or modulation of antennal position during visually guided flight maneuvers. To gain insight into the putative role of visual input in antennal positioning during flight, we filmed and quantified the antennal position of tethered flying moths in response to open-loop translational and rotational visual stimuli. We calculated the maximum change (ΔLA and ΔRA for left and right antennae, respectively) in position of each antenna in response to
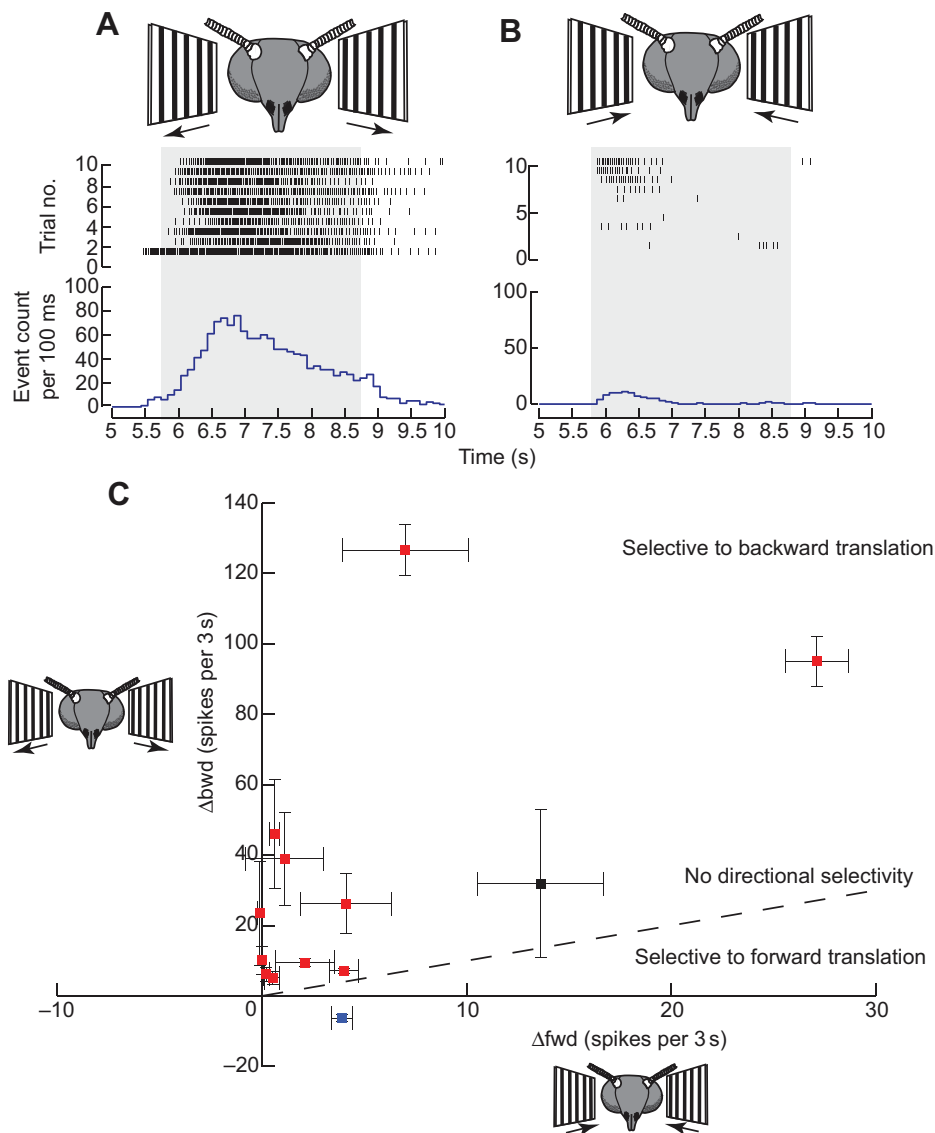
Fig. 3. Antennal muscle responses to visual translation. (A,B) Schematic diagram of the stimulus protocol (top), representative spiking raster plots (middle) and peri-stimulus time histograms (bottom) of intrinsic muscle EMG recordings from a single moth in response to open-loop backward (A) and forward (B) translational motion. These histograms use a 100 ms bin size for visual purposes. The gray shaded area represents the duration of the stimulus. There was a clear directional preference for backward translation in this case. (C) Plot of the mean change in firing rate during forward translation (Δfwd, average of 10 trials per moth) versus the mean change in firing rate during backward translation (Δbwd; N=13). Each point on the plot represents EMG data from a single moth, and error bars represent s.e.m. Muscle responses showing no directional sensitivity (black points) lie along a line with a slope of 1 (black dashed line), while those significantly biased toward backward (red points) or forward translation (blue points) lie above and below the line, respectively. Responses from 11 out of 13 moths showed a significant (P<0.05) directional preference for backward translation, whereas only one case showed a bias toward forward translation.

visual stimuli. A negative value of ΔLA or ΔRA meant that the antenna was brought forward, whereas positive values indicated caudal motion of the antenna.

Tethered flying moths moved both antennae in response to visual stimulation, typically through angles of more than 10 deg. The responses to open-loop translational motion were of three types: in some cases, the moths brought both antennae forward (Fig. 5A,B), whereas in others one antenna moved forward but the other moved backward (Fig. 5C, N=7). Responses to translation did not show a statistically significant handedness, as calculated from ΔRA–ΔLA (see Materials and methods for a detailed description). The values of ΔRA–ΔLA in response to backward and forward translation were also not significantly different from each other (Fig. 5D). The two antennae thus showed similar responses to translational motion, without directional selectivity.

In contrast to translational movements, antennomotor responses to open-loop rotation showed sensitivity to the imposed direction of rotation (Fig. 6A,B). Moths given a counterclockwise rotation always brought the right antenna forward, whereas the left antenna was either moved forward or backward (Fig. 6C, N=9). Similarly, during clockwise rotation, the left antenna was always brought

forward and the right antenna was either moved forward or backward (Fig. 6C, N=9). Responses to counterclockwise rotation were significantly skewed toward the right antenna, while responses to clockwise rotation were significantly skewed toward the left antenna (P<0.05). ΔRA–ΔLA values of these responses were also significantly different from each other (P<0.05; Fig. 6D). Thus, antennal responses to rotation were directionally selective, with greater forward movement of the antenna contralateral to the turn direction.

DISCUSSION
We show that visual feedback influences antennal position using both neurophysiological and behavioral experiments. Using EMG recordings, we found that the antennal muscles receive bilateral motion-sensitive visual input at time scales of 1–2 wing strokes, which is much slower than mechanosensory input from the Böhm's bristles (<1 wing stroke). This motion-sensitive input to the antennal muscles is also directionally selective. In the behavioral experiments, we presented open-loop motion stimuli to tethered flying moths and found that they modulate their antennal position in response to the visual stimuli. When provided with rotational
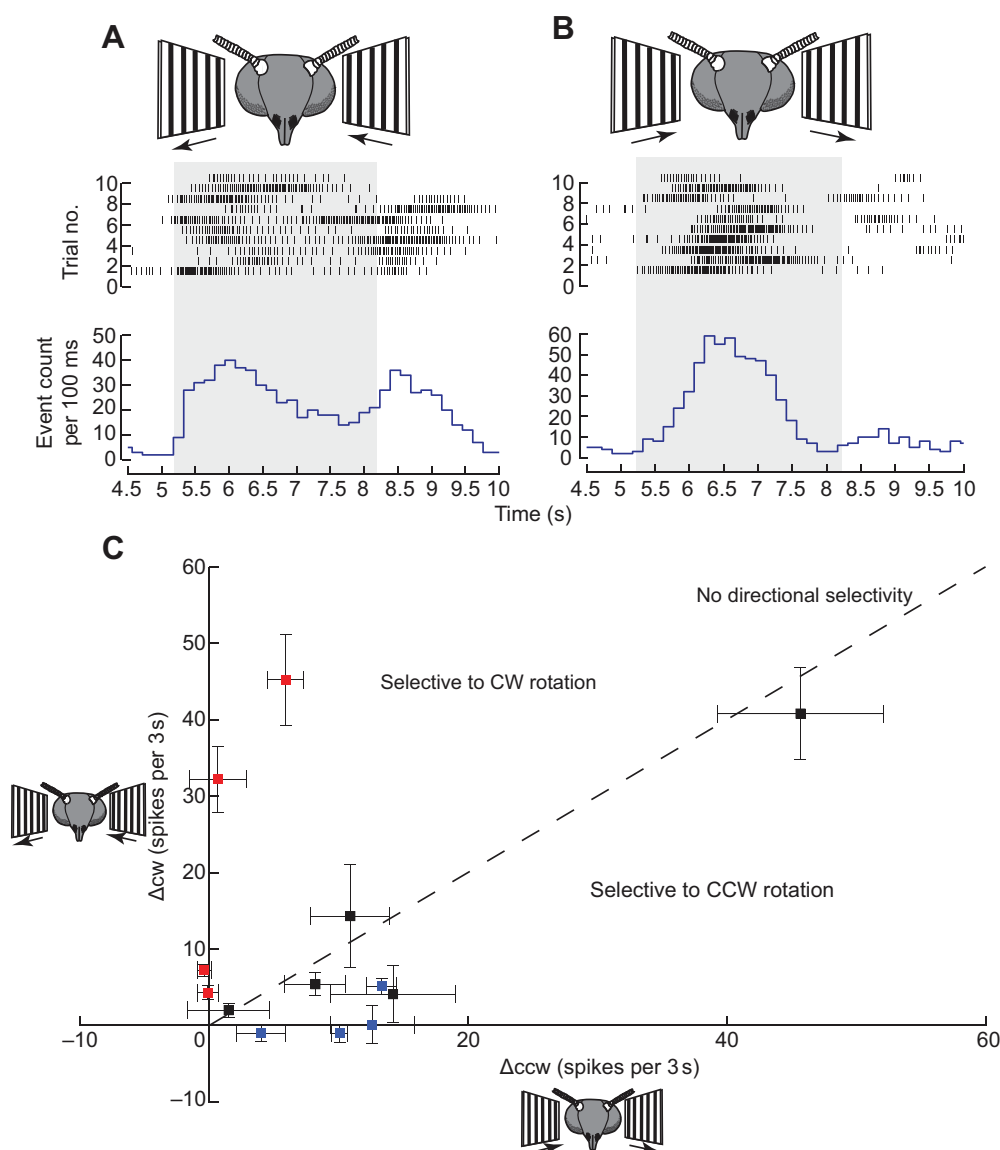
Fig. 4. Antennal muscle responses to visual rotation. (A,B) Schematic diagram of the stimulus protocol (top), spiking raster plots (middle) and peri-stimulus time histograms (bottom) of intrinsic muscle EMG recordings from a single moth in response to open-loop clockwise (A) and counterclockwise (B) rotational motion (stimulus represented by gray shaded area). The histograms use a 100 ms bin size for visual purposes. In this case, although the firing pattern in A shows distinct phasic components compared with that in B, the firing rates within the shaded region for the two directions of rotation were statistically similar. (C) Change in firing rate during counterclockwise rotation (Δccw) plotted versus the change in firing rate in response to clockwise rotation (Δcw) (N=13). Four of the observed responses showed a significant response bias to counterclockwise rotation (blue) and four to clockwise rotation (red); five showed no significant directional selectivity (black).

stimuli, the antennal positioning response showed directional selectivity, similar to the underlying neural circuitry.

Bilateral motion-sensitive visual input to the antennal muscles

In Orthopteroid insects which use their antennae as tactile sensors, moving objects elicit a tracking response from the ipsilateral but not the contralateral antenna (Honegger, 1981; Ye et al., 2003). However, antennal muscles of hawk moths responded to stimulation of both eyes (Fig. 1). This also contrasts with the mechanosensory input to the same muscles, which is thought to be ipsilaterally confined (Krishnan et al., 2012). In addition, we also observed that the latencies of muscle responses to contralateral and ipsilateral visual stimulation were similar (Fig. 2A,B). Motion-sensitive visual interneurons with directionally selective responses have been shown to arborize bilaterally in a region of the deutocerebrum (the posterior slope, dorsal lobe or AMMC, antennal motor and mechanosensory center) [moths (Wicklein and Strausfeld, 2000); bees (Hertel and Maronde, 1987)], which also contains the dendritic arbors of antennal mechanosensory and motor neurons (Kloppenburg, 1995; Maronde, 1991). It is therefore likely that circuits conveying

ipsilateral and contralateral visual information to the moth antennae may also contain similar numbers of neurons. This would enable the simultaneous acquisition of inputs from both eyes to synchronize antennal movements.

Our EMG recordings of antennal muscle responses to bilateral motion stimuli also showed that visual input to the antennae is sensitive to motion with significant directional selectivity (e.g. Fig. 3A,B). However, we did not observe responses of the antennal muscles to unilateral motion stimuli (supplementary material Fig. S3), which may indicate that input from both eyes is required for initiating antennal muscle responses. During these EMG recordings, we obtained multiple types of response to motion stimuli from the Ms-pp muscle, differing in the directional selectivity of the response. Open-loop rotations elicited three different types of muscle response: in some cases, the responses were preferentially biased toward counterclockwise rotation, and in others, they showed selectivity for clockwise rotation. Several responses did not show any overall directional bias (Fig. 4C). Open-loop translation mostly elicited responses selective for backward translation, although one case showed selectivity for the opposite direction of motion (Fig. 3C). The antennal muscles thus appear to receive directionally
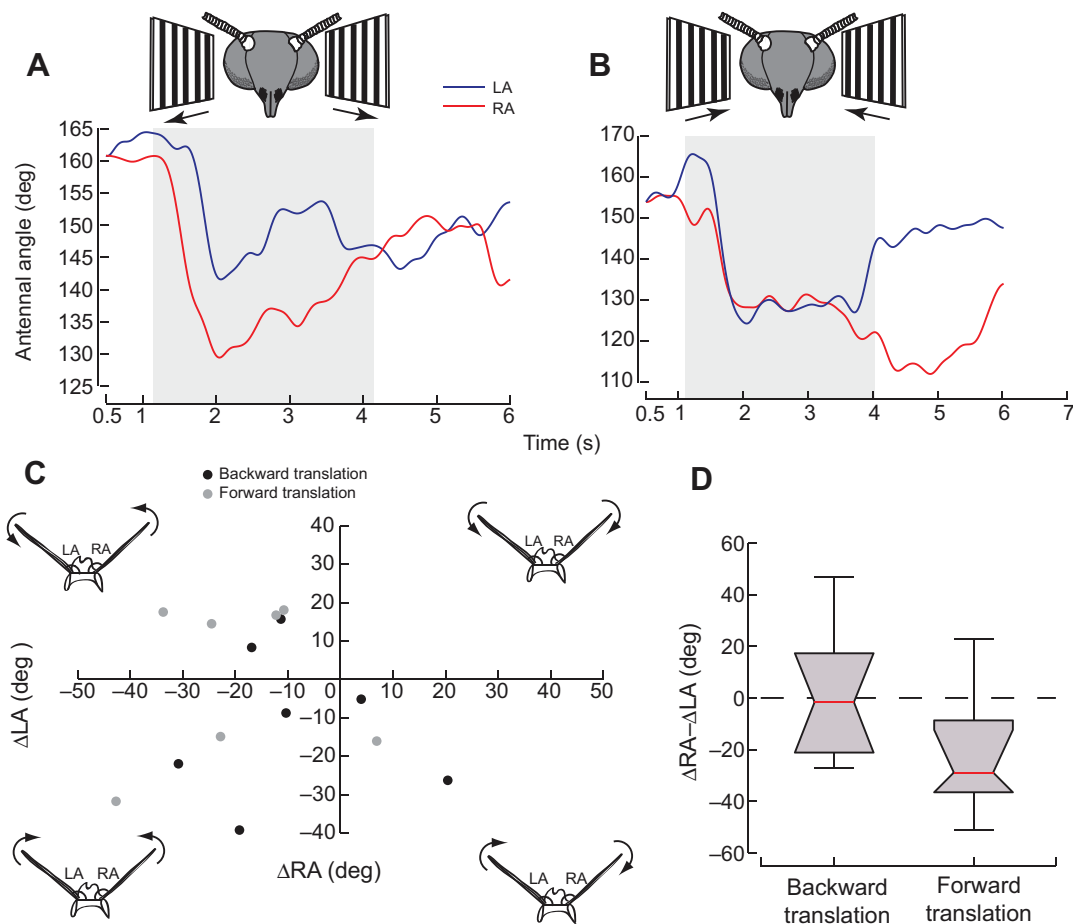
Fig. 5. Movements of the antennae in response to translation. (A,B) Representative behavioral responses of the left (LA, blue) and right antennae (RA, red) of tethered flying moths to open-loop backward (A) and forward (B) translational motion. The traces are normalized such that the first points on both are equal, to enable easy comparison. Although both show a greater forward movement of the right antenna, this was not true of all cases. (C) Change in right antennal angle (ΔRA) plotted versus change in left antennal angle (ΔLA) during forward (gray) and backward (black) translation. Negative values of ΔLA (negative *y*-axis) or ΔRA (negative *x*-axis) indicate that the antenna was brought forward, whereas positive values indicate backward movement. The schematic insets in each quadrant depict antennal movement directions for a point within that quadrant. (D) Asymmetry in the relative responses of the two antennae was determined by calculating ΔRA–ΔLA. Negative values indicate that the right antenna was brought forward to a greater extent, and positive values indicate a greater forward motion of the left antenna. Equal responses of the two antennae would result in a value indistinguishable from zero. There was no significant asymmetry or directional selectivity of antennal responses to translational motion (*N*=7).

selective motion input from multiple visual interneurons. Indeed, in both moths and bees, multiple motion-sensitive visual interneurons arborize in the deutocerebrum, with some even arborizing bilaterally (Wicklein and Strausfeld, 2000; Hertel and Maronde, 1987). Bees, like moths, show spatial overlap between the arbors of antennal sensory afferents and those of antennal motor neurons (Kloppenburg, 1995). The neural mechanisms of antennal positioning are thus likely to be similar in the two insects.

Role of antennal visuomotor responses in flight
Insects such as hawk moths typically fly at low light levels, which challenge the temporal acuity of their visual system (Theobald et al., 2010). In these insects, mechanosensory information from the antennae is crucial for flight control (Sane et al., 2007). In flies, visual input mediates relatively slow flight maneuvers while mechanosensory input from halteres is more important at high angular velocities, for which the gain of visual input to the flight motor is lower (Sherman and Dickinson, 2003; Sherman and Dickinson, 2004). Latency of visual input to the antennal muscles is 35–60 ms (or 1–2 wing strokes; wing beat frequency of *Daphnis*

nerii ~33 Hz), considerably slower than mechanosensory input from the Böhm's bristles (Fig. 2C), which activate the antennal muscles in <10 ms (Krishnan et al., 2012). Together, these data suggest that the reflex mediated by the Böhm's bristles provides rapid corrective feedback to the antennal muscles, whereas visual input may modify antennal position over longer time scales.

Tethered flying moths show changes in antennal position when provided with moving visual input. Rotational stimuli elicited directionally selective antennal responses, with a greater forward movement of the antenna contralateral to the turn direction. However, antennal movements did not show directional selectivity in response to translational motion. This contrasts with our EMG data from the Ms-pp intrinsic muscle, which almost always responded preferentially to backward translation. However, the results of these experiments are not directly comparable, as the antenna was fixed during EMG recordings, which may have altered mechanosensory feedback to the antennal muscles. The primary purpose of these experiments was to demonstrate the presence of motion-sensitive input to the antennal muscles, while the behavioral experiments served to demonstrate that this input could modulate antennal position. In addition, it is likely
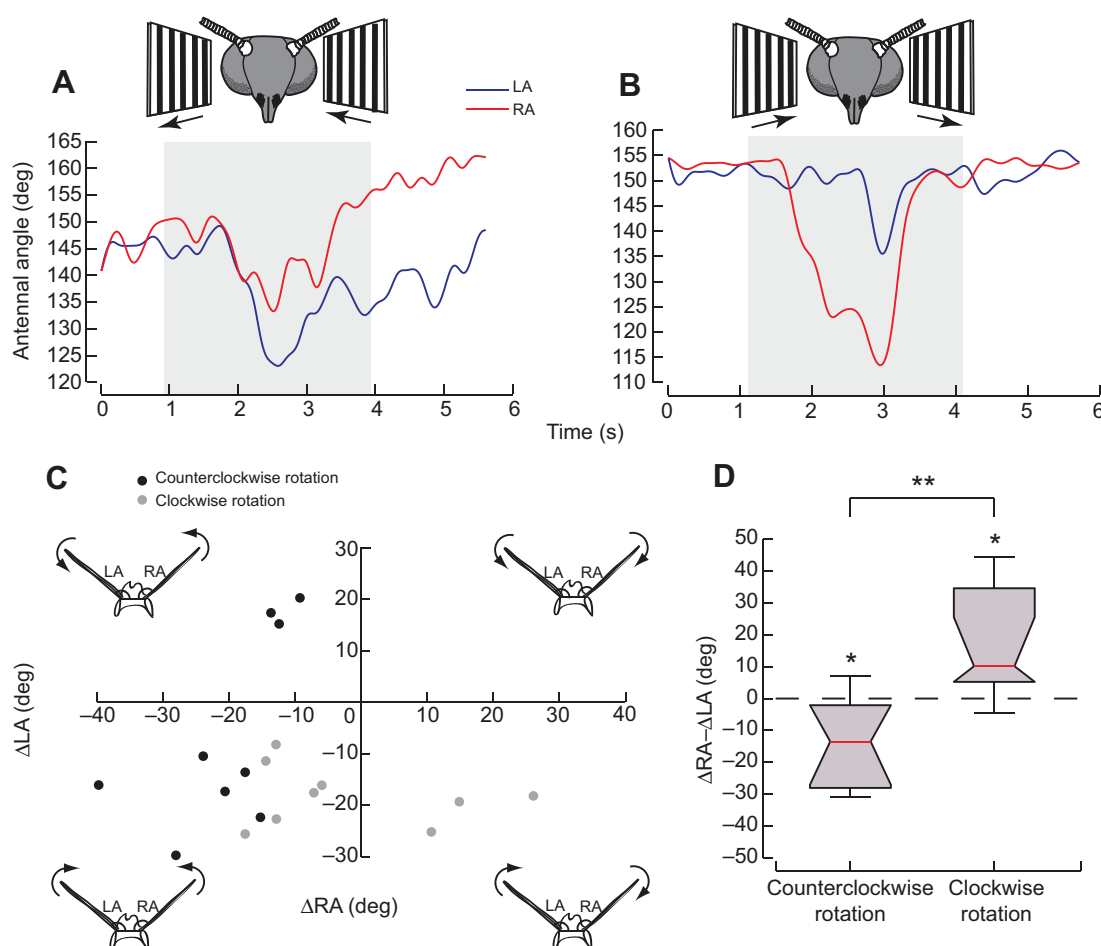
Fig. 6. Movements of the antennae in response to rotation. (A,B) Representative responses of the antennae to open-loop clockwise (A) and counterclockwise (B) rotational motion. Clockwise rotation induced greater forward movement of the left antenna (LA, blue), while counterclockwise rotation induced greater forward movement of the right antenna (RA, red). (C) Change in right antennal angle (ΔRA) plotted versus change in left antennal angle (ΔLA) during clockwise (gray) and counterclockwise (black) rotation. (D) Open-loop rotational motion resulted in antennal responses with both asymmetry (*$P<0.05$) and directional selectivity (**$P<0.05$) ($N=9$). Antennal responses to rotation thus involved a greater forward movement of the antenna contralateral to the turn direction.

that the combinatorial influence of other antennal muscles is also important and this will be the subject of future studies.

Our data suggest that a summation of visual inputs may occur within the antennal motor neurons during visually guided maneuvers. Information from both eyes triggers bilateral directionally selective responses of the antennae. The fruit fly *Drosophila melanogaster* actively moves its antennae in a direction opposite to the visual field motion. These visually induced changes in antennal position are thought to influence the acquisition of mechanosensory input by the Johnston's organs (Mamiya et al., 2011), in addition to the direct role of visual input in flight stabilization (Götz, 1968). In tethered flying moths, open-loop rotational stimuli elicited similar movements only of the antenna contralateral to the turn direction. This antenna was typically brought further forward than the other. However, the fact that such diverse insects show visuomotor responses of the antenna in flight, in spite of the differences in antennal architecture, suggests a general role for these responses in flight. The Johnston's organs situated in the pedicel–flagellum joint are thought to provide equilibrium-related cues during flight (Sane et al., 2007), and antennal positioning may thus enable Johnston's organs to unambiguously acquire these inputs (Krishnan et al., 2012). Modulating antennal

position during visually guided maneuvers may enhance the ability of the Johnston's organs to provide rapid information about self-motion. In *Drosophila*, changes in antennal position are accompanied by changes in the wing beat amplitude (Mamiya et al., 2011). Also, in hawk moths, stimulating the antennal muscles results in changes in flight parameters (Hinterwirth et al., 2012). Visually induced movements of the antennae may also be important in preventing collision of the antenna and the wing during turns. Such wing–antennal collisions are frequently seen in moths with ablated Böhm's bristles, which are unable to position their antennae properly (Krishnan et al., 2012).

The multisensory basis of antennal positioning in insects

Insects use external sensory cues to position their antennae in both flight and non-flight contexts. Honeybees maintain antennal position in relation to airspeed during flight (Heran, 1957). Cockroaches use a combination of mechanosensory input from scapal hair plates (Okada and Toh, 2000; Okada and Toh, 2001), and visual input during antennal tactile sensing (Ye et al., 2003). Honeybee antennae show definite movements in response to olfactory, visual and mechanosensory stimuli (Erber et al., 1993; Erber and Schildberger, 1980).
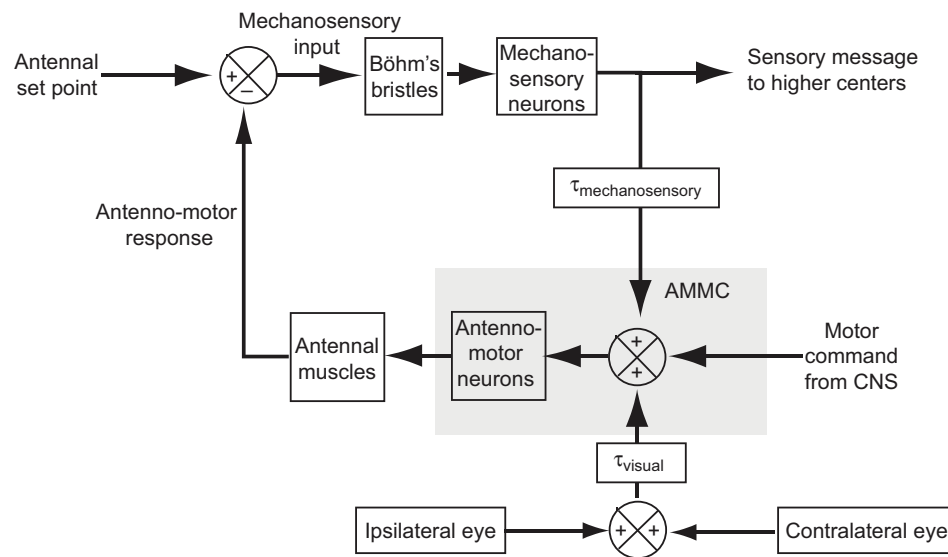
Fig. 7. Working hypothesis for antennal positioning as a function of multimodal sensory feedback. According to this model, the Böhm's bristles provide rapid feedback about changes in antennal position at sub-wing stroke time scales ($\tau_{mechanosensory}$), and correct deviations of the antennae by generating a motor response via a negative feedback loop (e.g. Krishnan et al., 2012). In addition, visual input from both eyes feeds into the antennal motor neurons, and may be important in slower (τ_{visual}; 1–2 wing stroke time scales) modulations of antennal position during visually guided flight maneuvers. To the best resolution of our data, the latency of the antennal response to visual input from the two eyes is the same. Hence, this is shown as a combined latency for the two eyes rather than as a separate latency for each eye. Visual and mechanosensory inputs are combined in the antennal motor and mechanosensory center (AMMC, gray shaded area) to generate the appropriate motor response of the antennae. Information about antennal position may be important for other sensory functions of the antennae and thus may be passed on to other regions of the brain.

Our findings from this and a previous paper (Krishnan et al., 2012) suggest that antennal positioning in flying hawk moths is a multimodal reflex involving at least two sensory modalities. On the shorter (<1 wing stroke) time scales, the Böhm's bristles provide rapid sensory feedback to maintain the antennal position during flight. However, during visually guided maneuvers such as turns, both eyes convey motion information to the antennal muscles within 1–2 wing strokes. The visual and mechanosensory inputs may combine to modulate antennal position in a context-dependent manner, as depicted in the preliminary model shown in Fig. 7. For example, the directional selectivity of responses to rotation can result from combined inputs of the two eyes at the antenna contralateral to the direction of turn, but weaker interaction at the other antenna. Combined visual and antennal mechanosensory inputs also mediate the abdominal flexion response in the hawk moth *Manduca sexta* (Hinterwirth and Daniel, 2010a). It remains to be seen whether antennal muscles are capable of detailed encoding of visual pattern movement, and finer-scale modulations of antennal position.

Antennal visuomotor responses have also been observed in other flying insects [in non-flight contexts (Erber and Schildberger, 1980; Honegger, 1981; Ye et al., 2003); in flight (Mamiya et al., 2011; Hinterwirth and Daniel, 2010b)]. This suggests that the multisensory control of flight antennal position may be a conserved feature in insects. Indeed, vision and mechanosensation may not be the only sensory inputs influencing antennal positioning. The antennal muscles of honeybees have been shown to receive bilateral olfactory input over much longer time scales of tens of wing strokes (Suzuki, 1975). In both hawk moths and honeybees, the motor neurons innervating these muscles arborize in the AMMC of the deutocerebrum (Kloppenburg, 1995; Kloppenburg et al., 1997). In crickets, the deutocerebrum contains arbors of descending interneurons, which convey both visual information and inputs from antennal mechanosensors to the ventral nerve cord (Gebhardt and

Honegger, 2001). This information may be involved in mediating responses of the flight motor as well as abdominal flexion during flight maneuvers in moths (Hinterwirth and Daniel, 2010a). Thus, the AMMC merits investigation as a putative center of multimodal sensorimotor integration in diverse antenna-mediated behaviors.

MATERIALS AND METHODS
Laboratory culture of hawk moths
All experiments described here were performed on laboratory-bred adults of the Oleander hawk moth, *D. nerii*. We maintained adult moths in Plexiglas chambers with their host plants, and collected eggs after 2–3 days. The larvae were reared on host plant leaves (*Nerium oleander* and *Tabernaemontana divaricata*) in mesh-topped boxes (see Krishnan et al., 2012). Pupae were embedded in sawdust until eclosion.

EMG recordings
Adult hawk moths were cold anesthetized and immobilized in a sawn-off syringe tube using molten dental wax. After 1 h of recovery, we positioned the moth under a swiveling dissection microscope and immobilized the left antenna by gluing the flagellum in a glass capillary tube. We used cuticular landmarks on the scapal surface to locate the Ms-pp intrinsic antennal muscle (Niehaus and Gewecke, 1978), and inserted a tungsten recording electrode (5 μm diameter, 2 MΩ impedance; FHC Inc., Bowdoin, ME, USA) into this muscle through the scape. A ground electrode was inserted into the frontal head capsule. To minimize the influence of extraneous visual cues, we performed all experiments in the dark with no light source except for the visual cue presented by the experimenter. Responses were amplified 1000× using a P55 AC differential extracellular pre-amplifier (Grass Instrument Co., W. Warwick, RI, USA). We used a custom interface written in LabVIEW (National Instruments, Austin, TX, USA) to acquire data at a sampling rate of 10 kHz via an analog to digital converter (National Instruments USB 6229).

Visual stimuli
We delivered 10 ms pulses of white light using a 1 W LED and measured the antennal muscle response to visual stimuli (Fig. 1A–E). The light source was placed 5 cm away from each eye, and directed onto the eye using a

cylindrical tube made of black paper. Another piece of black paper placed longitudinally between the eyes further blocked light from stimulating the contralateral eye. The intensity of light incident on the eye was approximately 356 lx.

To provide motion stimuli to the insect, we used a MATLAB routine (MathWorks Inc., Natick, MA, USA) from the Psychophysics toolbox (Brainard, 1997; Kleiner et al., 2007; Pelli, 1997). This routine generated a grating pattern that could be moved either backward or forward at a temporal frequency of 5 cycles s^{-1}, and a spatial frequency of 0.25 cm^{-1} [values based on data published elsewhere (O'Carroll et al., 1996)]. We used a computer equipped with a dual-VGA graphics card (nVIDIA GeForce 9800GT) to display patterns simultaneously on two monitors (Beetel 8×16 in LED monitors at 640×480 resolution, 60 Hz refresh rate). As determined by filming their output at 1000 frames s^{-1}, these monitors did not exhibit flicker at the temporal frequencies to which the visual systems of hawk moths are tuned (O'Carroll et al., 1996). The monitors were placed on either side of the insect at a horizontal distance of 14 cm from the eye, covering a field of view of ~105 deg for each eye.

We provided four different open-loop motion stimuli to the moths, each for the duration of 3 s. Throughout the paper, these stimuli are named according to the visual perception they were meant to create for a tethered moth. Thus, during 'forward translation', the stripes on both screens moved backwards (i.e. in an anterior-to-posterior direction), whereas during 'backward translation', the stripes moved forward (Fig. 3A,B, schematic diagrams). We also provided a 'counterclockwise rotation', for which there was clockwise movement of the visual field, and 'clockwise rotation', where the visual field moved counterclockwise (Fig. 4A,B, schematic diagrams). At the start and stop points of pattern motion, a pulse was sent to the analog-to-digital converter via the computer's sound card. We used this pulse to keep track of the stimulus start point.

EMG analysis
To generate raster plots of the responses, the raw EMG data were imported into pCLAMP 10.0 (Axon Instruments, Union City, CA, USA). These data were band-pass filtered offline (low cut-off 20 Hz, high cut-off 700 Hz; supplementary material Fig. S1) to remove movement artifacts and sorted using the thresholding functions of pCLAMP 10.0 to obtain a matrix of spike peak times. To calculate response latencies to white light stimuli, we binned the data from each moth (40–60 trials of each stimulus per moth) into histograms with 1 ms time bins. We normalized these histograms by dividing all values by the peak firing rate (such that the peak had a value of 1), and calculated the mean firing rate over the first 3 s of each plot, including the stimulus phase. The 'latency to significant firing shift' (i.e. at which the firing rate crossed 5 s.d. from the mean) and the 'latency to peak firing rate' were obtained from the normalized histograms of change in firing rate (see Krishnan et al., 2012).

To quantify directional bias in antennal muscle responses to motion stimuli, we calculated the average change in firing rate in response to open-loop backward (Δbwd) and forward (Δfwd) translation, and open-loop counterclockwise (Δccw) and clockwise (Δcw) rotation for each moth. These values were calculated by finding the average difference over 10 trials between the number of spikes fired during the 3 s stimulus and the number of spikes fired during the 3 s immediately before the stimulus (in spikes per 3 s). Given the variability of motor responses in the antennal muscle, quantifying the changes in mean firing rate before and during stimulus presentation provided the most objective criteria to statistically compare across all experiments. We compared Δfwd with Δbwd, and Δccw with Δcw using Welch's t-test to determine the statistical strength of the directional bias. For example, when Δfwd was significantly higher than Δbwd (P<0.05), the muscle response was said to show a directional preference for forward translation.

Tethered flight experiments and digitization of antennal motion
We cold anesthetized ~1 day old adult male moths, and tethered them ventrally to an aluminium post (2 mm diameter, 5–6 cm length) using a mixture of cyanoacrylate glue and sodium bicarbonate (Sane and Jacobson, 2006). A black spot was marked about 0.5 cm from each antennal tip for easy digitization. After dark-adapting the moths for 2 h, we filmed them in

tethered flight using two synchronized overhead high speed cameras (Phantom v7.3, Vision Research, Inc., Wayne, NJ, USA) at a frame rate of 100 frames s^{-1} (100 μs exposure). We provided these moths with both open-loop translational and rotational motion stimuli using two LED monitors as described above. In these experiments, the monitors were at a horizontal distance of 14 cm from the moth, but placed in a V-configuration such that the insect faced the apex. All experiments were performed under red illumination (>620 nm) to minimize extraneous visual cues, and the cameras were calibrated before and after the experiments.

We digitized the antennal tips and bases and obtained their three-dimensional Cartesian coordinates using a custom-written MATLAB code (Hedrick, 2008). After converting these into spherical coordinates, we calculated the angle made by the left (LA) and the right (RA) antennae with the line joining the two antennal bases.

Analysis of antennal responses
We calculated the magnitude of the maximal change in left and right antennal angles (ΔLA and ΔRA, respectively) from the mean pre-stimulus baseline in response to moving visual stimuli. Anterior (forward) movement of the antennae decreased the value of LA and RA, and therefore ΔLA and ΔRA were negative (Fig. 5C, Fig. 6C). We quantified asymmetry of the antennal response by calculating ΔRA–ΔLA for each animal in response to translational or rotational motion stimuli. A negative value indicates either that the right antenna was brought further forward than the left antenna, or that the right antenna moved forward but the left antenna moved backwards. Conversely, a positive value indicates a greater forward movement of the left antenna compared with the right antenna. We tested whether antennal responses were significantly asymmetric by comparing the mean ΔRA–ΔLA with a hypothetical mean of zero for each dataset (one-sample Student's t-test). We also compared the values of ΔRA–ΔLA for forward translation versus backward translation and counterclockwise versus clockwise rotational motion using the two-sample Student's t-test to determine whether the antennal visuomotor responses were directionally selective.

Author contributions
A.K. and S.P.S. conceived and designed the experiments, A.K. performed the experiments and analysed the data, and A.K. and S.P.S. interpreted the data and wrote the manuscript.

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